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Flexibility and Stretching Physiology:
Responses and adaptations to different stretching intensities

Tese elaborada com vista à obtenção do Grau de Doutor em Motricidade Humana na Especialidade de Biomecânica

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Flexibility and Stretching Physiology:
Responses and adaptations to different stretching intensities

Fisiologia da Flexibilidade e Alongamento:
Respostas e adaptações perante diferentes intensidades de alongamento

Abstract

Research and reported literature regarding the conceptual, methodological, and training effects of stretching with different intensities are scarce. The purposes of this thesis were to: i) explore and develop methodological conditions to achieve the second purpose (studies: 1 to 3); ii) characterize the acute and chronic effects induced by different stretching intensities on skeletal muscle and joint mechanical properties (studies: 4 to 9). Nine studies were conducted with total sample size of 257 participants. The findings of the methodological studies allowed to determine and improve the assessments of passive knee extension torque-angle (study 1), perception of stretching intensity through a new scale developed (study 2), and biceps femoris long head (BF) architecture using ultrasonography (study 3). The acute responses to stretching were seen to be different depending on the stretching intensity, and distinct mechanical responses were observed for either the joint or the muscle (studies 4 to 8). In respect to long-term effects of stretching, it was observed in an 8-week high intensity stretching training pilot study that intervention changed the BF architecture and increased joint maximal range of motion (study 9). Stretching intensity it is a valuable training variable that should be considered in intervention and research contexts.

Key-words: acute, adaptations, chronic, deformation, duration, flexibility, intensity, joint, length, passive torque, passive tension, perception, skeletal muscle, stiffness

Sumário

A investigação e literatura reportada sobre aspectos conceptuais, metodológicos, e os efeitos da intervenção do alongamento com diferentes intensidades são escassos. Os propósitos desta tese foram: i) explorar e desenvolver condições metodológicas para alcançar o propósito seguinte (estudos 1 ao 3); ii) caracterizar os efeitos agudos e crónicos induzidos por diferentes intensidades de alongamento ao nível das propriedades mecânicas da articulação e do músculo (estudos 4 ao 9). Nove estudos foram conduzidos, envolvendo um total de 257 participantes. Os resultados dos estudos metodológicos permitiram determinar e desenvolver modos em aceder ao `momento passivo-ângulo articular´ na extensão passiva da perna (estudo 1), à percepção da intensidade de alongamento através de uma nova escala criada (estudo 2), e à arquitetura da longa porção do bicípite femoral (BF) (estudo 3). As respostas agudas observadas demonstraram ser diferentes face à intensidade de alongamento, e distintas respostas mecânicas foram obtidas entre a articulação e o músculo (estudos 4 ao 8). Face aos efeitos de longo termo induzidos pelo alongamento, foi observado que uma intervenção de 8 semanas com intensidade de alongamento elevada revelou alterar a arquitetura muscular do BF e aumenta a amplitude articular passiva máxima na extensão da perna (estudo 9). A intensidade de alongamento é uma importante variável de treino que deve ser considerada em contextos de intervenção e de investigação.

Palavras-chave: agudo, adaptação, articulação, comprimento, crónico, deformação, duração, flexibilidade, intensidade, momento passivo, músculo esquelético, percepção, rigidez, tensão passivo.

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List of Acronyms

AME – Absolut method estimation

AUC – Area under the curve

BF – Biceps femoris long head

CG – Control group

CT – Constant torque

DC – Dissipation coeficient

ED – Energy dissipated

EFOV – Ultrasound panoramic extended field-of-view

ES (*d*) – Effect size

EMG – Surface electromyography

FA – Fascicle angle

FL – Fascicle length

HISD – High-intensity and short-duration

ICC – Intraclass correlation coefficient

LILD – Low-intensity and long-duration

MA – Muscle architecture

MDD – Minimum detectable difference

mROM – Maximal range of motion

MT – Muscle thickness

MTJ – Muscle-tendon junction

MVC – Maximal voluntary contraction condition (study 8)

MVIC – Maximal voluntary isometric contraction

no-MVC – No maximal voluntary contraction condition (study 8)

NRI – Non-rest interval stretching

OS – Onset of stretching perception

PNF – Proprioceptive neuromuscular facilitation

PT – Peak torque

P50 – Stretching at 50% of maximal tolerable torque– Stretching at 75% of maximal tolerable torque

P100 – Stretching at 100% of maximal tolerable torque

R - Repetition

RMSE – Root mean square error

ROM – Range of motion

RI – Rest interval stretching

RO40 – 40% of torque from the onset of stretching perception to maximal tolerated torque range

RO60 – 60% of torque from the onset of stretching perception to maximal tolerated torque range

RO80 – 80% of torque from the onset of stretching perception to maximal tolerated torque range

R40 – 40% of maximal tolerated torque (study 4) or range of motion (study 7)

R60 – 60% of maximal tolerated torque (study 4) or range of motion (study 7)

R80 – 80% of maximal tolerated torque (study 4) or range of motion (study 7)

R100 – Stretching at maximal range of motion

r – Pearson

SEM – Standard error of measurement

SG – Stretching group

SIS – Stretching intensity scale

SP – starting position

SR – Stress relaxation

SSI – Supersonic shear imaging

ST – Semitendinousus

T-A – Torque-angle curve

VAS – Visual analog scale

VL – Vastus medialis

Introduction

Static stretching is a common practice in sports, physical therapy, and wellness for purposes of flexibility improvement (Baechle & Earle, 2008; Page, 2012; Ratamess, 2011). The reasons for its use are related to physical performance enhancement, injury prevention, rehabilitation, and well-being (McHugh & Cosgrave, 2010; McNeal & Sands, 2006). Here, a considerable research has been conducted in the last years focusing on the flexibility assessment and the acute and chronic physiological effects induced by static stretching (Ichihashi, Ibuki, & Nakamura, 2013).

The human flexibility has been examined *in* vivo through the measurement of the angle and the torque during the joint passive motion (Magnusson, Simonsen, Aagaard, & Kjaer, 1996), and the muscle-tendon stretching using the ultrasonography imaging (Kubo, Kanehisa, & Fukunaga, 2002), and more recently using the supersonic shear wave imaging (SSI) (Bercoff, Tanter, & Fink, 2004; Maïsetti, Hug, Bouillard, & Nordez, 2012). A varied type of protocol tests and methodological approaches has been used across researchers to analyze the acute and chronic effects on passive torque-angle induced by stretching (Ichihashi et al., 2013). However, due to the different methods across studies, it is difficult to compare and interpret the results. For instance, the testing protocol for the same joint varies and consequently the mechanical stress imposed to the body is different (Figure 1). Also, the different torque-angle outcomes are obtained among studies without reporting upon methodological errors and reliability outcomes (see the section 1.1 Passive torque-angle assessment on page 19). Thus, is important to determine the reliability of the outcomes in a systematic testing protocol to assess the joint passive torque-angle, in order to use in intervention studies.

A considerable number of stretching studies have focused in the knee flexors, especially on the hamstring muscle groups (Chan, Hong, & Robinson, 2001; Magnusson et al., 1996; Rushton & Spencer, 2011). The hamstring is composed by three muscles: semitendinosus, semimembranosus, and the biceps femoris (short and long head). In the last years, a focus has been given by some researchers on the biceps femoris long head (BF), because this muscle is a common site of injury (Timmins, Porter, Williams, Shield, & Opar, 2014), and is related to physical tasks (McCormack et al., 2014). The muscle architecture has been examined in the hamstring muscles because it relates to muscle functional properties, as force-length and force-velocity relationship. However, few studies have focused on the adaptations of BF architecture to stretching using ultrasonography (Kwah, Pinto, Diong, & Herbert, 2013; Lima, Carneiro, Alves, Peixinho, & Oliveira, 2014). In addition, the previous studies that assessed *in vivo* the BF architecture have not fully reported the reliability of the architecture outcomes and the ultrasound assessment procedure (Kwah et al., 2013). For instance, it is unknown what methodological error that should be considered to determine adaptations of the BF to intervention studies (e.g. physical training). Thus, in order to analyze the effects of stretching on BF architecture the reliability and assessment errors should be determined first.

The stretching interventions are often determined by setting together two training variables: duration and intensity. Such variables constitute a topic of continued interest among researchers (Ichihashi et al., 2013). Previous studies have investigated which "dose" of stretching would give the greatest results on maximal range of motion (ROM), capacity to tolerate the joint passive torque, and the passive torque at a given submaximal angle (Ichihashi et al., 2013). However, most of these studies gave attention to the stretch duration (Matsuo et al., 2013; Ryan et al., 2008), and few have investigated the intensity (Behm & Kibele, 2007; Walter, Figoni, Andres, & Brown, 1996; Young, Elias, & Power, 2006). For instance, we are just aware of three studies that investigated the stretching intensity, and none have examined the effects on joint passive torque-angle (Behm & Kibele, 2007; Walter et al., 1996; Young et al., 2006). Since the intensity is a common variable used to set the static stretching "dose", it is important to explore the effects of stretching intensity on joint mechanical properties. Moreover, the instruments, methodological procedures, or a theoretical model to assess the stretching intensity are scarce. In clinical, physical training, and research contexts, the stretching intensity is often determined based on the participant's perception to stretching using pain or discomfort thresholds as criteria for maximal ROM (McHugh & Cosgrave, 2010). For determination of submaximal stretching intensities, the percentage of stretching perceived exertion has been use (Behm & Kibele, 2007) or the percentage of maximal ROM (Walter et al., 1996). However, the previous methods used to assess the stretching intensity are not consistent (McHugh & Cosgrave, 2010), and the relation between the perception of stretching intensity and the physiological responses to stretching (e.g. joint torque) has not been explored. For instance, it is unknown if the participants rate the stretching intensity according to the degree of resistance to stretch or the degree of range of motion. Also, it is unknown how the participants perceived the stretch intensity when performing passive maneuvers above the maximal ROM obtained in a first repetition. Additionally, there is not a valid and reliable instrument to assess the perception of stretching intensity. Such an instrument would be helpful to measure the stretch intensity in intervention studies, such as a stretching training program.

The studies that focus on the acute effects induced by stretching have previously examined the passive joint torque-angle (Magnusson et al., 1996) and the muscle-tendon mechanical response (Nakamura, Ikezoe, Takeno, & Ichihashi, 2012). The observations were usually taken during, immediately after, or through the time course until some point after the stretching. However, many issues are still to be explored for the different time observations. For instance, during the stretching it is unknown if resting between repetitions would provide greater results on maximal ROM and peak torque increase, when stretching to the maximal ROM in each repetition. The previous research suggests that not resting between repetitions would provide a higher stretching efficiency (Duong, Low, Moseley, Lee, & Herbert, 2001). In addition, it is well known that during the static stretching a decrease of the joint passive torque occurs along the time (McHugh et al., 1992; Magnusson et al., 1995), and this is often called stress relaxation (SR). It has been showed that the muscle also relaxes *in vitro* (Abbott & Lowy, 1956), and it is expected to relax *in vivo* (McHugh et al., 1992; Magnusson et al., 1995) when exposed to static stretching through a passive tension decay during the time. However, the previous studies observing the muscle stress relaxation have been based on the torque-angle measurements, and to our knowledge no previous study has assessed *in vivo* the muscle SR with more direct measurements. Due to recently imaging technological advances, the muscle passive tension can be assessed *in vivo* using SSI with a higher validity and reliability (Bercoff et al., 2004; Maïsetti et al., 2012). Consequently, the conclusions of previous studies examining *in vivo* the muscle SR are still to be confirmed. For instance, it was concluded in a previous study that the relative muscle SR during stretching is independent of its length, although this conclusion was based on torque-angle measurements (Tian, Hoang, Gandevia, Bilston, & Herbert, 2010). Another issue not fully explored is regarding the structural adaptations underlying the mechanical joint response during the static stretching. Using an ultrasound assessment, a previous study has concluded that muscle length increases during the SR, after observing a muscle-tendon junction displacement during the static stretching (Nakamura, Ikezoe, Takeno, & Ichihashi, 2013). Because only one study has been done on this topic, and it was not clearly described the protocol procedures (i.e. blinding data analysis), the previous conclusion should be confirmed by assuring appropriate methodological procedures.

The previous studies on the immediate effects induced by stretching have also not fully explored different issues. Here, two questions are emergent to be answered. The first regards immediate joint mechanical effects induced by stretching with: 1) different intensities and durations, and 2) different intensities and equal durations. The previous studies examining stretching with different intensities and durations often compare protocols with inverse proportion of the stretch intensity and duration variables (Dempsey et al. 2010; Light, et al. 1984; Moriyama et al., 2013; Steffen & Mollinger, 1995; Usuba et al., 2007). In other words, protocols are often compared for high intensity & long duration to low intensity & short duration in order to determine which variable “intensity” or “duration” would induce the greatest effects on joint mechanical properties. However, the studies results are not consistent and it lacks studies on joint passive torque-angle properties of healthy people. Thus, it is unknown if stretching with higher intensity and short duration would produce the same joint mechanical effects, than a lower intensity and long duration stretching. Regarding the joint mechanical effects of stretching for different intensities and equal durations, we do not know the existence of previous studies. Also, we are unaware of studies that have attempted to investigate the effect of different durations in different intensities. Thus, these questions still remain to be answered.

The second question on the immediate effects induced by stretching, relates to the physiological variables underlying the acute adaptations after performing static stretching. It has been very common in previous studies, the use of the joint passive torque and angle measurements to infer about the passive force-length relation of the muscle-tendon unit, in the absence of significant muscles contractile activity determined by surface electromyography (EMG) (Weppler & Magnusson, 2010). However, the relation between the joint passive torque and muscle-tendon unit passive tension has never been examined *in vivo* during passive slow maneuvers. Thus, it is unknown if the passive torque measurements reflect the muscle passive tension.

In respect to the timecourse effects on joint and muscle passive properties induced by static stretching, most of the previous studies have examined the effects within an hour since there is evidence that effects return to baseline after this time (Figure 4, page 33). Previous studies have observed different timecourse effects across stretching protocols with different durations, and have concluded that the joint passive torque decrease at a given angle is greater for longer stretching durations (Matsuo et al., 2013; Ryan et al., 2008). However, the effects induced by different intensities have not been studied, for either equal stretching durations or for different stretching durations. Thus it is also important to know what effects are produced by different stretching intensities on either passive joint torque-angle and muscle-tendon properties.

Regarding the long-term effects induced by stretching, a considerable number of previous studies have been conducted to observe the mechanical adaptations of the joint through the assessment of passive torque-angle during stretching, but few have examined the structural effects on muscle-tendon unit (Lima et al., 2014; Nakamura et al., 2012). The previous studies observing the long-term effects on the joint torque-angle, often discuss this parameter as a way to represent the muscle passive force-length in order to realize if the muscle length increases together with maximal ROM as a consequence from continued stretching training. Although there is no relation established between the joint and muscle measurements, the conclusions across studies are not consistent. Some studies support that maximal ROM gains are accompanied by a decrease of passive torque at a given length, and therefore they infer that muscle length increases as a consequence of torque decrease (Chan et al., 2001; Guissard & Duchateau, 2004; Kubo, Kanehisa, Kawakami, & Fukunaga, 2001; Marshall, Cashman, & Cheema, 2011; Nakamura & Ikezoe, 2012). One study observed an increase of torque after a stretching program (Gajdosik, Allred, Gabbert, & Sonsteng, 2007). Nevertheless, most of the studies found no changes in passive torque at a given angle despite the increase of maximal ROM (Folpp, Deall, Harvey, & Gwinn, 2006; Harvey et al., 2003;

Magnusson, Simonsen, Aagaard, Srensen, & Kjaer, 1996). Consequently, they argued that increase in maximal ROM was due to an increase in the capacity to tolerate the joint torque (i.e. stretch tolerance), and not because of structural changes in tissues (Weppler & Magnusson, 2010). However, some studies examined the muscle architecture (and thus measuring the fascicle length) after the stretching training did not observed changes compared to baseline (Lima et al., 2014; Nakamura et al., 2012). These studies lasted less than 4- and 8-weeks, and were not tested for different stretching intensities, only for duration. Because connective tissue adapts within a longer time compared to muscle (McClure, Blackburn, & Dusold, 1994), and the mechanical stimulus (i.e. intensity and duration) might have been insufficient (Lima et al., 2014; Nakamura et al., 2012), maybe adaptations did not occurred on muscle architecture. Indeed, some authors mention the stretching intensity and duration as a key factor for muscle structural adaptations (Jacobs & Sciascia, 2011), since there is a small evidence that a high intensity stretching provides higher gain in maximal range of motion (Walter et al., 1996). Thus, because of the importance of muscle architecture in performance and injury prevention, it should be sought to know what is the effect of a high intensity stretching on muscle architecture parameters.

The present thesis aimed to extend the knowledge about acute and chronic effects induced by stretching with different intensities and durations. Nine studies were conducted for the aim of this thesis: three studies (1 to 3) aimed to explore and develop methodological conditions to the follow studies; three studies (4 to 6) were designed to analyze the acute mechanical effects on the joint induced by stretching with different intensities and durations; three studies (7 and 8) were conducted to analyze muscle response during and after stretching with different intensities; and one pilot study (9) meant to determine the chronic effects induced a high intensity stretching training on muscle architecture.

A – Review of literature

The stretching and flexibility have been investigated for different purposes and using different methodologies, when examining the acute and chronic effects induced by stretching on muscle and joint mechanical properties. This chapter examines briefly the previous studies on methodological procedures used to assess the muscle and joint mechanical properties, the definition and assessment procedure for determination of stretching intensity, and the acute and chronic effects induced by stretching on muscle and joint mechanical properties.

1. Joint mechanical properties

1.1 Passive torque-angle assessment

In the last three decades, the human flexibility has been studied through the measurement of joint passive torque and angle during stretching maneuvers in both clinical (Bjorklund et al., 2001; Carvalhais et al., 2011) and research contexts (McHugh et al., 1992; Knutson et al., 2000; Hoang et al., 2005; Nordez et al., 2006; Gombatto et al., 2008) in the absence of significant muscle activity. Isokinetic dynamometry has been commonly used for joint torque-angle measurements to examine the acute and chronic effects of stretching (Gajdosik, 1991; Magnusson et al., 1995; Chan et al., 2001; Potier et al., 2009; Nordez et al., 2006; Rushton & Spencer, 2011; McHugh et al., 2012). During the stretching maneuver it is normal to observe an exponential passive torque increase (i.e. dynamic phase), and a logarithmic torque decrease along the time at a static stretching position due to the joint stress relaxation (i.e. static phase) consequently of the viscoelastic properties of the tissues crossing the joint. Here, different mechanical outcomes have been used for analysis to examine the effects of stretching (Figure 1).

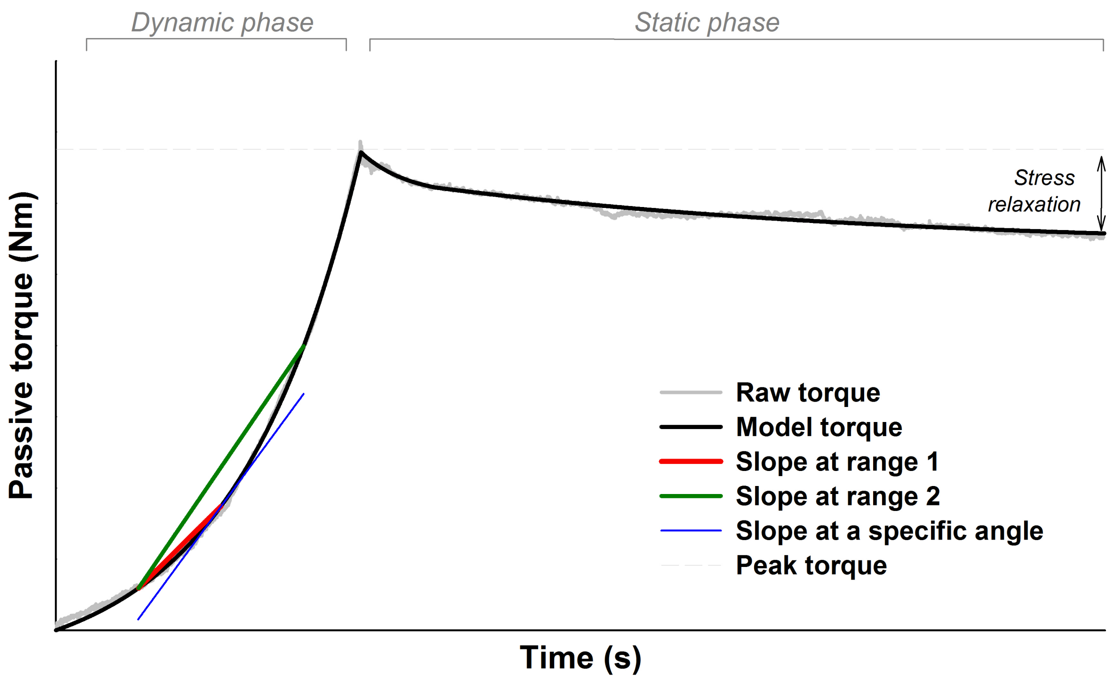

Figure 1. Schematic joint passive torque-angle response to stretching and outcomes often analyzed during the dynamic (i.e. when the joint is mobilized) and static (i.e. when joint is fixed in a static angle) stretching phases, for both raw (gray line) and modeled data (black line). The lines red, green, and blue represent the slope of the torque-angle curve in the dynamic phase obtained in a small range of motion, higher range of motion, and a specific angle of the curve, respectively.

The torque-angle assessments have been performed for different joints (Carvalhais et al., 2011; Gombatto et al., 2008; Knutson et al., 2000), and different populations (Magnusson, 1996; Reid & McNair, 2010). However, there is a lack of standardization and absence of information on methodological errors of these protocols leading to difficulties in comparing results across studies. In addition, the previous studies have reported the reliability for assessing the joint passive torque-angle but have used different approaches. For instance, there are studies reporting the assessment reliability by comparing the torque at the maximal ROM (Gajdosik, Linden, & Williams, 1999; Magnusson et al., 1995), at a constant torque (Harvey, Byak, Ostrovskaya, & Glinsky, 2003), or in different percentages of maximal ROM (Gombatto et al., 2008); or by comparing the slope at different points of the torque-angle curve (Nakamura, Ikezoe, Takeno, & Ichihashi, 2011). In addition some of the studies only reported the Pearson correlation coefficient (*r*) as a reliability outcome (Magnusson et al., 1995), and do not clarify if the analysis was performed for a value at a certain point or all range of the torque-angle curve. Consequently, the intraclass correlation (ICC) and *r* coefficients for the different torque-angle parameters vary across studies. Thus, the intra- and inter-session reliability of different torque-angle parameters it still remains to be determined.

Moreover, the misaligning the dynamometer and the joint axes on torque-angle response, which is often noted as a study limitation (Magnusson et al. 1995; Chan et al., 2001; Hoang et al., 2005; Gombatto et al., 2008; Herda et al. 2012). This is noted as a limitation because it affects the measurement of the joint angle and passive torque. However, no previous study has examined the extent effect of this misaligning on torque-angle measurement. Consequently, the impact of such misaligning on torque-angle response remains unknown.

1.2 Knee extension testing protocols

Previous studies have assessed the passive torque-angle during the stretching in different joints. For instance, the ankle (Morse, Degens, Seynnes, Maganaris, & Jones, 2008), knee (S. Magnusson et al., 1996), hip (Carvalhais et al., 2011), wrist (Knutson et al., 2000), spine (Gombatto et al., 2008), and elbow (Herbert & Gandevia, 1995) have been investigated in past studies. The knee passive extension torque-angle assessments has been used by some researchers in order to infer about injury and human locomotion performance issues (Chan et al., 2001; Gajdosik, 1991; Magnusson et al., 1995; McHugh et al., 2012; Nordez et al., 2006; Potier et al., 2009; Rushton & Spencer, 2011). However, these studies have varied with regard to methodology (**Figure 2**), and have failed to control for factors that can affect the measured torque. For instance: foot and head position during testing, since the position of these body segments affect the resistance to passive knee extension (Læssøe & Voigt, 2004; McHugh et al., 2012); pelvic girdle stabilization by positioning the contra lateral thigh in the maximum extension possible, and avoiding thigh rotation (Herda, Costa, Walter, Ryan, & Cramer, 2012; Magnusson et al., 1995; Nordez et al., 2006), because there is evidence that pelvic girdle easily moves when the hip is flexed and consequently this affects force measurement (Bohannon, Gajdosik, & LeVeau, 1985); and the clamping force applied to body segments during stabilization, specially in the thigh, since this factor affects the longitudinal tension of the tissues being deformed (Rushton & Spencer, 2011). Failure to consistently control for these factors may result in differences in passive knee torque-angle relationships, which may affect reliability.

Figure 2. Different passive knee extension torque-angle testing protocols used in past studies: A) McHugh et al. 2010 (Magnusson et al. protocol); B) Chan et al. 2001; C) Rushton & Spencer, 2011. All images were reprinted with permission.

1.3 Outcomes interpretation

The previous studies have used the joint torque-angle measurements (Figure 1) to infer about the viscoelastic properties of the muscle-tendon unit in different conditions. The elastic property has been investigated by measuring the passive torque at given angle (Magnusson et al., 1996), or by determining the slope at a certain point of the torque-angle curve or in a curve range (McHugh, Kremenic, Fox, & Gleim, 1998; Nakamura et al., 2011). The viscous property has been examined by stretching the tissues surrounding the joint with different velocities through the joint mobilization with varied joint angular velocities (McNair, Hewson, Dombroski, & Stanley, 2002; Nordez, McNair, Casari, & Cornu, 2009), and by analyzing the torque response during when the joint is held in a static stretching position (i.e. stress relaxation) (Neto et al., 2013; Tian et al., 2010). The ROM has been often considered as a muscle length outcome, and passive torque as reflecting the muscle passive tension (Weppler & Magnusson, 2010). Consequently, previous researchers have estimated the muscle stiffness based on torque and angle relationship (Gajdosik, 2001), or by comparing the passive torque at a given joint angle (Magnusson et al., 1996), but some previous researchers have defined this relationship not as a stiffness muscle parameter, but instead as a related variable (i.e. flexibility index) (Kubo et al., 2002). Although this relationship may not represent muscle stiffness (Baumgart, 2000), various criteria have been used to define this parameter. For example, the slope of the torque-angle curve has been determined at different sites of the torque-angle curve. For instance, the slope was determined by Herda et al. (2012) at the maximal ROM, by Magnusson et al. (1996) in the three curve ranges, by Ryan et al. (2008) in different angles near the maximal ROM, and by McHugh et al. (1998) in a specific ROM range (i.e. 20 to 50° joint angle range). The maximal passive torque obtained at the maximal ROM (i.e. peak passive torque) has been considered an outcome to represent the human tolerance to stretch (Magnusson et al., 1996). In a static condition, the torque decline during static stretching has been interpreted as a tissues viscoelastic property to relax under mechanical stress (McHugh et al., 1992; Taylor, Dalton, Seaber, & Garrett, 1990).

In addition, the joint passive torque-angle has also been analyzing through the parameters of mathematical models fitting to the raw data (Hoang, Gorman, Todd, Gandevia, & Herbert, 2005; Hoang, Herbert, Todd, Gorman, & Gandevia, 2007). The values for the model parameters have been used as a representative outcome to all torque-angle curve range. However, it unknown if the reliability is similar for the different output parameters, such as torque values for various joint angles, slopes of the torque-angle curve for different ranges of angles, and parameters of mathematical models that fit to the raw torque-angle data (McHugh et al., 1992; Hoang et al., 2005; Nordez et al., 2006; Nakamura et al., 2011;

Magnusson et al., 1996; Herda et al., 2012). Thus, the most important and reliable output remains unknown.

2. Muscle properties assessment

2.1 Architecture

The muscle architecture has been examined in previous studies because it is related to muscle performance (Wakahara, Kanehisa, Kawakami, Fukunaga, & Yanai, 2013), functional task (McCormack et al., 2014), and to injury (e.g. strain) (Timmins et al., 2014). The reliable assessment of muscle architecture, including muscle thickness, fascicle length and fascicle angle is essential for scientific and clinical use in order to quantify alterations after acute (i.e. within-day) and chronic (i.e. between-day) exercise training, detraining and dietary interventions. Ultrasonography has been used reliably for the assessment of fascicle length, fascicle angle and muscle thickness *in vivo* in humans (Kwah et al., 2013; Thoirs & English, 2009) with the fascicles being relatively clearly delineated by the echoes from interspaces between them, which contain fatty and connective tissues and blood vessels (Blazevich, Gill, & Zhou, 2006; Noorkoiv et al., 2010). Whilst the information required for muscle thickness and fascicle angle measurements is easily obtained in a single ultrasound image, it is usual to use extrapolation techniques (Cronin, Carty, Barrett, & Lichtwark, 2011; Scott, Engstrom, & Loeb, 1993), image montage (Chleboun, France, Crill, Braddock, & Howell, 2001; Potier, Alexander, & Seynnes, 2009), or to use extended-field-of-view (Noorkoiv et al., 2010) to measure the length of fascicles that project off the imaging window (Figure 3). Such techniques may reduce measurement reliability because of the assumptions implicit to the techniques.

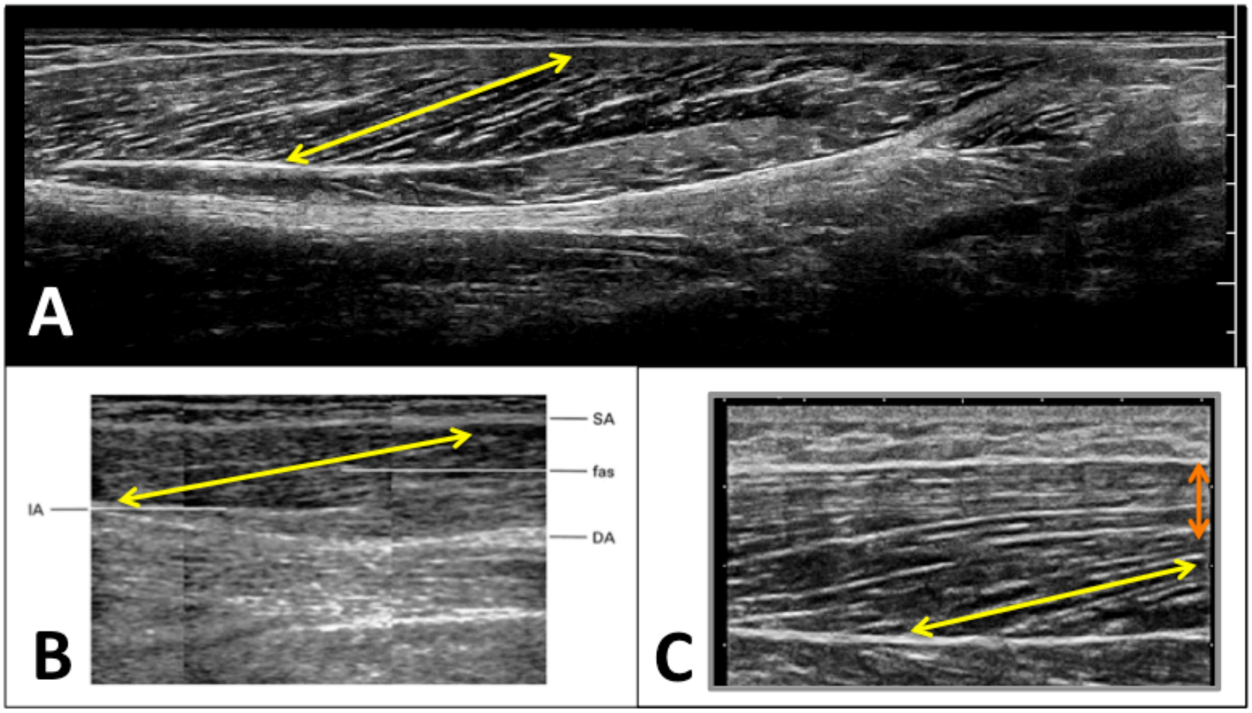

Figure 3. Ultrasound images for the biceps femoris long head architecture using different assessment techniques: A) Panoramic extended-field-of-view; B) image montage (image reprinted with permission from the Chleboun et al 2001); C) linear extrapolation technique.

Note: the yellow arrow follows the fascicle pathway observed in the image; and the orange arrow determines the height from the tracking fascicle to the superficial aponeurosis.

The reliability of muscle architecture measurements using ultrasonography has been seen to vary depending on the type of muscle, or procedure to assess the architecture parameters (Kwah et al., 2013). In a systematic review performed by Kwah et al. (2013) regarding ultrasound assessments on muscle architecture, it was reported a different range of reliability outcomes between muscles, and within the same muscle. For instance, the ICC for vastus lateralis fascicle length measurement ranged from 0.62 to 0.99, and the fascicle angle measurement ranged from 0.51 to 1.00. The number of examiners performing the image acquisition and image digitizing, and the width of the sonographic image was reported to explain the reliability assessment variance. Thus, the studies examining effects on muscle architecture after a certain stimulus (e.g. strength training) should always account for the specific methodological error regarding the muscle being assessed. However, not all the previous studies examining the muscle architecture reported the muscle reliability assessment outcome.

In addition, Kwah et al. (2013) indicated that studies assessing biceps femoris long head (BF) architecture did not report reliability statistics for their measurements (Chleboun et al., 2001; Kellis et al., 2009; Potier et al., 2009). Previous studies have shown that the BF architecture is related to muscle performance (Wakahara et al., 2013), functional task (McCormack et al., 2014), and to injury (e.g. strain) which is particularly incident to this muscle (Timmins et al., 2014). A recent systematic review (Kwah et al., 2013) indicated that studies assessing BF architecture did not report reliability statistics for their measurements (Chleboun et al., 2001; Kellis et al., 2009; Potier et al., 2009). Although two recent studies (Lima et al., 2014; Timmins et al., 2014) reported a high reliability for BF architecture measurements (ICC>0.78), without fully exploring the reliability of measurements and fully describing the procedures to assess BF architecture. For instance, it is not clear if assessments were performed by different examiners in both image acquisition and digitizing. Since the number of examiners may affect the reliability of the measures (Kwah et al., 2013), so it is important to determine to what extent the reliability of BF assessment is affected. They also did not distinguish the reliabilities to the different architecture parameters; and finally they do not calculate the minimal detectable change between test and retest measurements. Thus, is not known if: 1) sonographic assessment of BF architecture parameters is sufficiently reliable when assessed (image acquisition and digitizing) by the same examiner or different examiners, 2) whether the width of the imaging window critically influences measurement reliability, and 3) what is the minimal detectable change that falls outside the measurement error in the BF architecture ultrasound assessment by the same examiner or different examiners.

Biceps femoris long head morphology

The BF is a bi-articular muscle that crosses both knee and hip joints, and it is stretched during the knee extension and hip flexion. Cadaveric studies have reported that BF has a non-uniform fascicle length and angle, and it presents a mid aponeurosis that is visible in the muscle belly (Kellis et al., 2009). In the resting (non-contracted) condition, fascicles are curved and oriented in three planes and therefore difficult to visualize in their entirety in a single sonographic image using a conventional linear ultrasound probe. Moreover, the previous studies reporting a longitudinal mid-muscle aponeurosis, have mentioned that the aponeurosis extends from the proximal muscle-tendon junction to the distal tendon-junction, which is visible in ultrasound images and onto which superficial fascicles insert (Kellis et al., 2009) (Figure 3). However, this aponeurosis presents a non-linear path in the resting condition even though the superficial BF aponeurosis follows a linear path for most of length of the muscle belly. Moreover, the proximal and distal BF muscle-tendon junctions (MTJ) are different in shape (Figure 19, page 83). The distal BF MTJ is superficial, close to the skin, and its most distal point is easily observed, whereas the distal BF MTJ ends more proximally compared to the biceps femoris short head. At the proximal site, the BF MTJ is located deep and merges medially to the semitendinousus tendon, that together inserts onto the ischial tuberosity. Thus, the most proximal site of proximal MTJ is less visible than the distal MTJ.

2.2 Passive tension and stiffness

The measurement of the passive properties of human tissues *in vivo* has been a big challenge for researchers in the last years. However, to our knowledge, only one study has provided a method to measure *in vivo* the passive tension-length relationship of gastrocnemius during stretching maneuvers using a isokinetic assessment (Hoang et al., 2005; Hoang et al., 2007; Nordez et al., 2010). The muscle-tendon unit length was calculated based on cadaveric models, and its passive tension through torque-angle measurements of the ankle with the knee at different angles.

Moreover, due to recently advances in imaging technology, it has recently been shown that the shear elastic modulus measured using supersonic shear imaging elastography (SSI, Bercoff et al., 2004) is linearly related to the passive muscle tension, providing a non-invasive estimation of changes in passive muscle tension (Maisetti et al., 2012; Koo et al., 2013). Previous studies have reported that the assessment of muscle shear elastic modulus is reliable in resting (Lacourpaille, Hug, Bouillard, Hogrel, & Nordez, 2012) and in slow stretching (Maisetti et al., 2012) conditions. The quantification of the shear elastic modulus is based on the low frequency shear wave velocity of the tissue, after applying a mechanical stimulus through an acoustic radiation force impulse (i.e. "push") at different depths of the tissue (Bercoff et al., 2004; Gennisson et al., 2010). The velocity of the shear waves that mainly radiates in transverse directions is quantified through a supersonic scanning, and a estimation of elastic modulus are performed based on the assumption that the shear wave velocity increases in tissues of higher stiffness (Bercoff et al., 2004; Maïsetti et al., 2012). Compared to other elastography methods, the SSI has the advantage in presenting a quantitative elastic modulus outcome in a few miliseconds, without producing an external mechanical stimulus with the probe. However, this method still requires strict technical usage when assessing the tissue elastic modulus to obtain valid measurements (Bercoff et al., 2004; Gennisson et al., 2010). In other case, the SSI measurements may not have a physiological meaning (Akagi & Takahashi, 2013).

3. Stretching intensity

3.1 Definition

The stretching intensity has been poorly studied, and its definition it is not consistent among authors (McHugh & Cosgrave, 2010;) and recognized institutions (Baechle & Earle, 2008; Ratamess, 2011). For instance, the concept of stretching has been misunderstood with the concept of flexibility (Bandy, 2003; Magnusson et al., 1996). Some previous authors have used the term stretching to refer to a change in range of motion (Magnusson et al., 1996), and others have used the term flexibility to describe the change in muscle extensibility or length (Bandy, 2003). Also, previous studies examining the effects of different stretching intensities have determined the intensity below the maximal ROM differently (Behm & Kibele, 2007; Walter et al., 1996; Young et al., 2006). For example, Walter et al. (1996) and Young et al. (2006) have determined the intensity as a percentage of maximal range of motion, and Behm & Kibele (2007) determined the intensity as a percentage of the perceived exertion to stretching feeling pain. The wording for the description of stretching symptoms is also not consistent among studies, and consequently makes it difficult to confer consistency to the definition of stretching intensity. It is reported that the type of stretching symptom reported by persons being stretched varies (Boyd, Wanek, Gray, & Topp, 2009). For instance, Boyd et al. (2009) observed during a passive straight leg raise stretching maneuver that the participants of the study reported the term that best described the stretching symptom between “tension”, “tightness”, “ache”, “burning”, “pain”, or “stretch” sensation on the tissues being stretched, but the most frequent terms were “stretch” or “tension”.

While there is some variability in the definition of intensity, and how the concept is used, some authors have defined this variable in a manner with which we agree (McHugh & Cosgrave, 2010; Page, 2012). For instance, previous authors defined the stretching intensity as a change of the tissue length (e.g. muscle-tendon unit) and a consequent increase of its tension; and defined the flexibility intensity as a change in joint range of motion (Page, 2012). However, most of the previous studies have applied the stretching intensity concept using criteria related to the presence or absence of pain symptoms induced by stretching, since the setting of stretching intensity has been performed based on the tolerance to stretching reported by the participants (McHugh & Cosgrave, 2010; Page, 2012). In other words, the stretching intensity was controlled by subjective assessment of human tolerance to stretch using criteria of pain or discomfort thresholds. This indicates that human stretching intensity has mechanical and psychological components that should be interpreted together.

3.2 Maximal range of motion

The two main methodological considerations in determining the maximal ROM concerns the criteria used for setting the maximal ROM and the assessment reliability in performing the maximal ROM. The criteria with or without the use of related pain thresholds for defining the maximal range of motion has been inconsistent across studies (McHugh & Cosgrave, 2010). For instance, the pain thresholds used among studies has varied between “a mild discomfort” (Cramer, 2007), “just before the discomfort” (Unick, Kieffer, Cheesman, & Feeney, 2005), “point of discomfort” (Knudson, Noffal, Bahamonde, Bauer, & Blackwell, 2004), “point of discomfort but not pain” (Herda et al., 2012), “just before of the onset of pain” (Matsuo et al., 2013), “onset of pain” (Magnusson et al., 1997), “onset of soreness” (Cè, Margonato, Casasco, & Veicsteinas, 2008), and “tolerable pain” (Nelson, Kokkonen, & Eldredge, 2005).

Some other studies have performed stretching to the maximal ROM without using a painful related criteria and thus reporting that performed to the stretching to the "maximum tolerable passive torque threshold" (Ryan et al., 2010), "strong or moderate pulling sensation" (Boyce & Brosky, 2008), or until achieve "a sensation similar to a static stretching maneuver" (Magnusson et al., 1995). However, despite different criteria to achieve the maximal ROM, the inter-day reliability for producing the maximal ROM has been reported to be high when the using the same instruction between assessments (Branco et al., 2006; Gajdosik et al., 1999).

Regarding the inter-day reliability for determination of the maximal ROM using different types of criteria, we are just aware of one study that compared two different criteria (Branco et al., 2006). Branco et al. (2006) determined the maximal joint ROM in two different days by using two different instructions: 1) to stretch to the point of discomfort, and 2) to stretch to the point of pain. It was observed a high reliability for both instructions, and consequently the authors concluded that both stretching intensities could be determined using different pain related criteria

3.3 Perceived exertion & stretching

The human ability to rank mechanical perceived sensations has been evidenced in the past (Borg, 1998; Stevens, 1957). Based on this premise, several instruments (e.g. scales) have been developed and validated and to examine the perceived exertion for different physical capacities (Borg, 1998; Garcin, Wolff, & Bejma, 2004; Robertson et al., 2003). These previous studies were only conducted after first validating the relation between the perceived exertion and the physiological variables expressed during the respective physical practice. Thus, just under this validation is possible to study the perceived exertion to the specific physical exercise manifestation.

Muscle stretching is a practice spread worldwide in sports and in rehabilitation, and some studies have assessed for its intensity based on the tolerance to stretch (Bjorklund et al., 2001; Branco et al., 2006; Behm & Kibele, 2007). Here, tolerance to stretch may be interpreted as perceived exertion. However, to our knowledge, only one study has used an existing scale (i.e. CR10 Borg scale) to assess the stretching intensity (Bjorklund et al., 2001). The scale was used to compare the scale score after a 2-week static stretching program, at the same submaximal stretching intensity (i.e. for the same joint angle). However, the CR10 Borg scale has not been validating with the physiological variables expressed during the stretching. For the use of a scale to assess the perceived exertion during the stretching, it is first necessary to validate the scale score with the stretching physiological variables. In addition, the scale also should be composed of specific properties to measure the proposed variable in all possible intensity-range, and have an exclusive instruction to assess the physical exertion variable. In this context, the conceptual Borg's continuum model (Borg, 1998) has been used as a theoretical starting point for the development of constructs that establish the relationship between perceived exertion and physiological variables. According to Borg's model, physiological responses should be observed during physical performance. Thus, for a correct assessment of stretching intensity based on stretching perception using a scale, it must be confirmed first the relationship between the stretching perception and the stretching physiological variables.

The most studied stretching physiological variables are the joint passive torque and angle (Hoge et al., 2010; Kubo et al., 2003), and more recently the muscle passive tension (Maïsetti et al., 2012). When stretch intensity is increased, it is observed a joint passive torque rise because of the greater tissues tension (Maïsetti et al., 2012). However, the relation between the flexibility performance (i.e. ROM), joint mechanical response (i.e. torque), and the perceived exertion has never been studied. Thus, it remains to have a valid instrument to assess the perception of stretching intensity.

4 Static stretching effects

4.1 Acute

The acute effects of stretching are normally examined during the stretching (i.e. when the joint is fixed at a static position), immediately after the stretching, and for the timecourse effects. Such effects have been induced for different durations and stretching modes. In the follow sections we briefly review the literature for each testing time interval.

4.1.1 During

The main joint physiological variables studied during the static stretching are the ROM and the passive torque. When stretching is performed by a set of repetitions, the maximal ROM and the maximal tolerable torque are often analyzed across the repetitions. Once the stretching is held statically in a fixed joint angle, the torque decreases along the time due to tissue lengthening has been analyzed. The biological tissues undergo stress relaxation (SR) when exposed to a length beyond the slack length (Abbott & Lowy, 1956). Thus, when performing a passive static stretching a decrease in passive torque is observed in both healthy (McHugh et al., 1992; Magnusson et al., 1995) and pathological (Reid & McNair, 2010) populations. The previous studies have used the torque-angles measurement to infer about the muscle-tendon unit SR, and have concluded that different factors that affect the SR response. For instance, Sobolewski et al. (2013) reported that subjects with different levels of flexibility (i.e. maximal ROM) did not show differences in SR normalized to the peak torque. Neto et al. (2013) also observed that the relative SR values were similar between subjects with different body compositions. On the other hand, Gajdosik (2006) reported that

EMG surface activity of the muscles involved in the static stretch affects the SR response. Also, a previous study has observed that the relative SR was different between populations of different age (Sobolewski, Ryan, Thompson, McHugh, & Conchola, 2014). However, the SR that occurs during static stretching it is still a not fully understood (Duong et al., 2001; Magnusson et al., 1995; McHugh et al., 1992; Tian et al., 2010). For instance, it is unknown if the joint torque SR during the stretching reflects the SR of muscle-tendon unit. Also it is unknown to what extent the joint torque relaxation is affected by the muscle-tendon unit length. In a previous study of Tian et al. (2010), it was observed that the relative torque relaxation during a static plantar flexors stretch was poorly affected by the gastrocnemius length. The authors suggested two hypotheses to explain this result: i) the SR is mainly due to single-joint structures, or ii) SR is independent of the gastrocnemius length. If the second hypothesis is valid, which remains to be confirmed with more direct measurements (i.e. supersonic shear imaging), then this could have very interesting applications for relaxation modeling. It has recently been shown that the shear elastic modulus measured using supersonic shear imaging (SSI, Bercoff et al., 2004) is linearly related to the passive muscle tension, and thus providing a non-invasive method to estimate the changes in passive muscle tension (Koo, Guo, Cohen, & Parker, 2013; Maïsetti et al., 2012). Therefore, this method can extend the previous findings of the Tian et al. study and confirm whether or not the relaxation of the gastrocnemius muscle is independent from the muscle-tendon length.

From a practical intervention perspective, it is reported that physiological responses to physical exercise can be detected by the perceived exertion (Borg, 1998). For instance, the visual analog scale score (VAS) is related to joint angle and passive torque in stretching maneuvers (Freitas, Vaz, Bruno, Valamatos, & Mil-Homens, 2013). However, whether or not the perception of stretching intensity varies according the joint torque or muscle passive tension during the SR is unknown.

Another issue not fully explored relates to the anatomical responses that occur during the SR. A recent study (Nakamura, Ikezoe, Takeno, & Ichihashi, 2013) reported that muscle length increased during the SR, using ultrasound measurements performed on the myotendinous junction (MTJ) of the gastrocnemius. It was found a correlation between MTJ displacement and the torque decrease during the static stretch. Muscle lengthening during the SR would also mean that the tendon is shortened. It would imply that muscle is more viscous than tendon such that the SR is more due to muscle than tendinous structures. Considering that tendon contribute ~70% of the change in length of the passive gastrocnemius muscle tendon unit (Herbert et al., 2011), this result could be surprising and needs to be confirmed with more direct measurements on muscle fascicles.

In respect to the joint torque-angle responses among a set of stretching repetitions, the maximal ROM and tolerable passive torque have been reported to increase along the repetitions (Cabido et al., 2014).

However, the minimal number of repetitions to increase these outcomes has not been reported consistently across studies, and few studies have explored the number of repetitions for each stretching purpose (i.e. either increasing the maximal ROM or the tolerable passive torque) (Boyce & Brosky, 2008; Fowles, Sale, & MacDougall, 2000; Halbertsma, van Bolhuis, & Goeken, 1996; Herda et al., 2012; Magnusson, Aagard, Simonsen, & Bojsen-Møller, 1998; Mizuno, Matsumoto, & Umemura, 2011). For instance, regarding the maximal ROM gains, Fowles et al. (2000) reported a significant increase after one 135-s static stretching repetition for the ankle plantarflexors. Boyce et al. (2008) reported a significant ROM increase with only one 15-s stretching repetition for the knee flexors. Herda et al. (2012) observed a significant increase only at the fourth 30-s stretching repetition. In respect to the increase in the maximal tolerable passive torque gains, Mizuno et al. (2011) observed a significant maximum tolerable torque at the fifth repetition of 60-s static stretching for the ankle flexors. Halbertsma et al. (1996) reported an increase of maximal passive torque in a straight leg raise test only after 10 stretching repetitions of 30-s each for the hamstring muscle group. Magnusson et al. only observed an increase of maximal ROM at the third repetition with 90-s static stretching (Magnusson et al., 1998). As a consequence of different results of previous studies, the minimal number of repetitions to change either maximal tolerance to passive torque or ROM has still to be examined.

Moreover, from a training intervention point of view, previous studies have suggested that the highest degree of stretching should be performed to achieve a greater acute maximal ROM and decrease of the passive torque at a given angle (Cabido et al., 2014; Herda et al., 2012; Walter et al., 1996). For this purpose, the number of repetitions is increased as well the duration of the stretch, or specific methods are used [e.g. proprioceptive neuromuscular facilitation method (PNF) or the constant torque), to obtain the highest ROM in the stretching maneuver (Boyce & Brosky, 2008; Matsuo et al., 2013; Sharman, Cresswell, & Riek, 2006). However, it is believed by some authors that the increase in ROM across stretching repetitions are due to the torque relaxation that occurs during the stretch (Cabido et al., 2014; Herda et al., 2012). Nevertheless, the recovery of stress relaxation has been shown to be higher with longer rest intervals (Duong et al., 2001). In addition, the passive torque returns to baseline values at some point after the static stretch (Mizuno et al., 2011; Mizuno, Matsumoto, & Umemura, 2013). These facts suggests that the lack of rest interval would not allow a torque recovery after the stress relaxation period, and thus a higher ROM could be achieved in a subsequent stretching repetition. However, it is unknown if resting between repetitions potentiates the acute increase in ROM and the torque decrease. In addition, the PNF stretching is reported to be more effective for ROM gains, compared to a static stretching with rest intervals between repetitions (Magnusson & Simonsen, 1996; Sharman et al., 2006). However, the PNF do not use rest intervals between repetitions. Thus, it is unknown if a similar stretching procedure without muscle contraction (i.e. stretching without resting between repetitions) would also be more effective compared to a stretching with rest intervals.

4.1.2 Immediate

A common objective among studies examining the acute effects induced by stretching is to determine which dose of stretching would give the highest increase in ROM and tolerable torque, and a decrease of passive torque at a given angle. Most of these previous studies have tested for different stretching durations (Matsuo et al., 2013; Ryan et al., 2008), and a reduced number of studies have examined the intensity of the stretching (Walter et al., 1996; Young et al., 2006). However, among these intensity studies we are aware of just one study that examined the effect of intensity on the maximal ROM (Walter et al., 1996), and it was concluded that a higher stretching intensity induced a greater maximal ROM increase. The remaining intensity studies have investigated the stretching intensity together with the duration, in order to determine which variable (`intensity´ or `duration´) would provide greater results by comparing stretching with high intensity and short duration to low intensity and long duration with an inverse proportion (Dempsey et al. 2010; Light, et al. 1984; Moriyama et al., 2013; Steffen & Mollinger, 1995; Usuba et al., 2007).

Regarding the stretching duration, McHugh & Cosgrave (2010) have compared the results of some studies that used different durations, and concluded that the immediate decline of resistance to stretch was greater when stretching with a higher stretching duration (Figure 4). This conclusion is also supported by the results of Matsuo et al. (2013), that recently compared the effects of stretching for different durations. However, a similar conclusion was not obtained for the increase of maximal ROM.

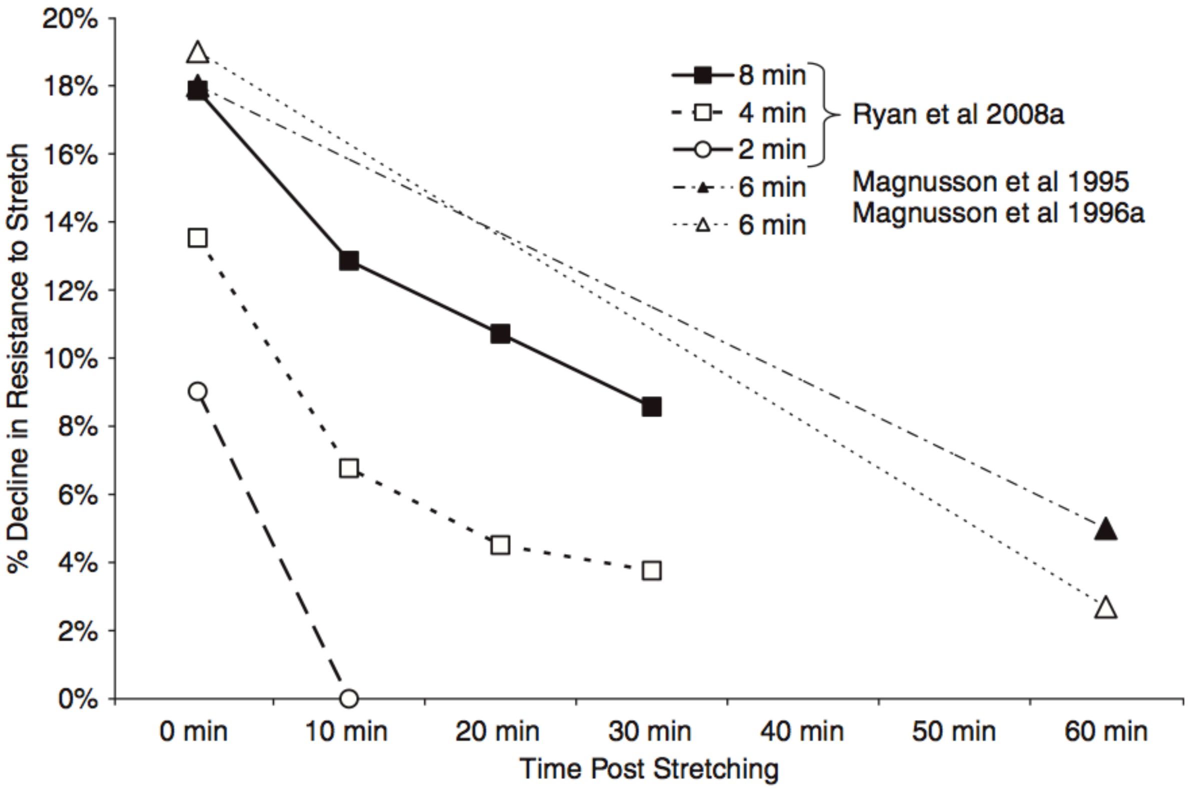

Figure 4. Comparison for the acute effect of different stretching durations within one hour from different studies on the resistance to stretch. Data are from the Magnusson et al. (1995), Magnusson et al. (1996), and Ryan et al. (2008) studies , and analysis was performed by McHugh & Cosgrave (2010). Figure reprinted with permission from McHugh & Cosgrave 2010.

In respect to the stretching intensity, as mentioned before, we are aware of just three studies examining the effects of stretching intensity (Behm & Kibele, 2007; Walter et al., 1996; Young et al., 2006). However, only one study investigated the effects of stretching intensity on maximal ROM gains, but no observation was performed for the joint passive torque response (Walter et al., 1996). The intensity was set as a percentage of maximal ROM (100%), and it was observed that the highest stretching intensity produced the greater ROM gains.

Regarding the studies that investigated the effects of stretching by testing together the intensity and duration, the results are not consistent (Dempsey et al. 2010; Light, et al. 1984; Moriyama et al., 2013; Steffen & Mollinger, 1995; Usuba et al., 2007), and few assessed the effects on joint passive torque in humans. For instance, Jacobs & Sciascia (2011) have argued stretch duration and intensity are inversely related, and that increasing stretch duration may be effective to change the T-A curve. Other authors have suggested low stretch load and duration will not be enough for joint flexibility increase, but duration should be prioritized rather than intensity (Light et al., 1984; McClure et al., 1994; Usuba et al., 2007). In contrast, other authors support the use of a higher intensity instead of duration to increase the joint flexibility (Dempsey et al., 2010; Moriyama et al., 2013; Steffen & Mollinger, 1995). However, no investigations have examined the T-A curve response in consequence of different stretch intensities. It is unknown if the stretch intensity and duration have the same impact on human joint passive T-A response. Hence, it is important to test these two variables together to understand the impact on T-A curve.

4.1.3 Timecourse

As described previously, the static stretching induces changes on maximal ROM, tolerance to stretch, and passive torque at given angle (Magnusson et al., 1996). However, these effects appear to be transient in time (Figure 4). For example, regarding the effects on passive torque at a given angle, Magnusson et al. (1996) reported that the decrement in passive torque after a five 90-sec static stretching repetitions recovered within 1-hour. Ryan et al. (Ryan et al., 2008) observed that passive torque in ankle dorsiflexion returned to baseline values within: 1) 10-min after a 2-min of static stretching, 2) and 20-min after a 4 and 8-min stretch. Mizuno et al. (2013) observed that ankle passive torque returned to baseline within 15-min, and the maximal dorsiflexion range of motion (ROM) was still increased until 60-min after stretching after 5-min static stretching to the calf muscles. The previous authors recently observed that ankle passive torque recovery occurred within 10-min after static stretching (Mizuno et al., 2013). However, these previous studies did not compare different stretch intensities. Instead, they have studied different stretch durations. To the best of our knowledge, no previous studies have investigated the timecourse effects for different stretching intensities, or different doses of combined stretching intensity and duration.

4.2 Chronic

Previous studies examining the chronic adaptations induced by stretching have often used the torque-angle measurements to determine if stretching chronically changes the muscle length, and thus explaining the maximal ROM increases after stretching training (Weppler & Magnusson, 2010). To conclude that muscle length increased as a consequence of the stretching training, the previous authors defined as a representative physiological response would be a decrease of the joint passive torque (i.e. reflecting muscle passive tension) for a given joint angle (i.e. reflecting the muscle length) after stretching, or the inversed observation (i.e. increased joint angle for the same joint torque load) (Figure 5).

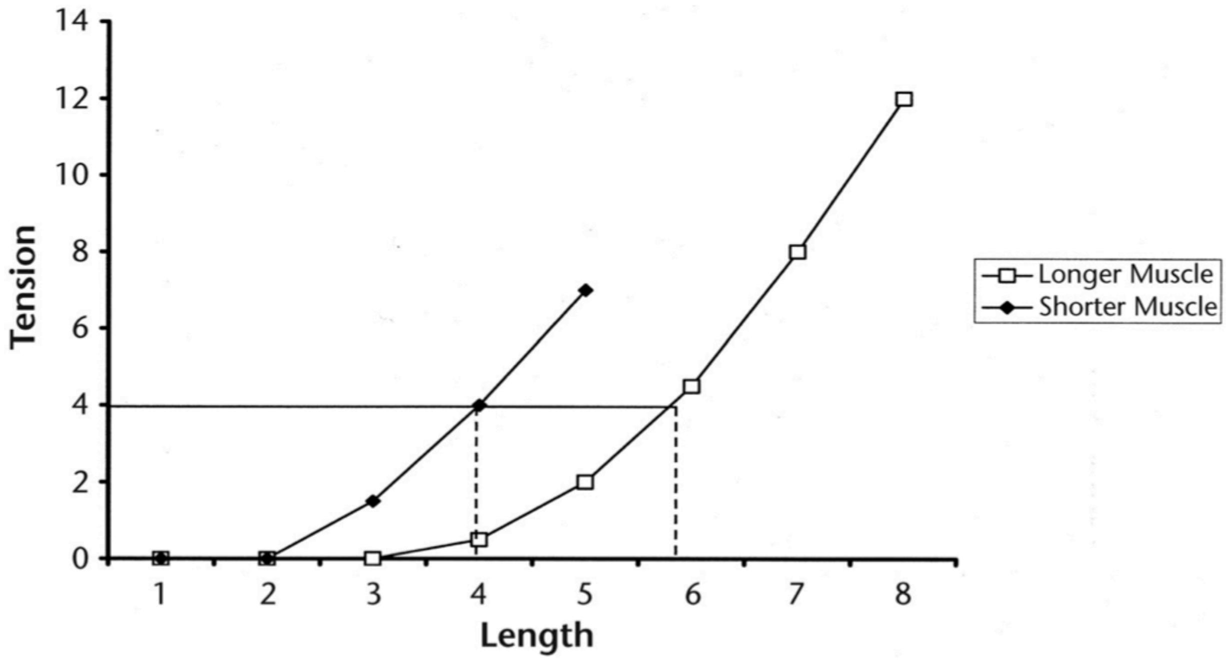

Figure 5. Muscle passive tension-length models of two different lengths to illustrate the mechanical difference that should be expected in the torque-angle relationship, assuming that the joint torque-angle response reflects the muscle passive tension-length. For the same muscle length (i.e. joint angle), the longer muscle should provide a less passive tension (i.e. joint passive torque). Figure reprinted with permission from Weppler & Magnusson 2010.

However, the conclusions of previous studies observing the long-term effects of stretching on torque-angle relationship have been inconsistent. For instance, in some studies it was found a decrease of passive torque at a given angle (Chan et al., 2001; Guissard & Duchateau, 2004; Kubo et al., 2001; Marshall et al., 2011; Nakamura & Ikezoe, 2012). One study has observed an increase of passive torque at a given angle (Gajdosik et al., 2007). However, most of the studies have found no changes in the passive torque at a given angle (Folpp et al., 2006; Harvey et al., 2003; Magnusson et al., 1996). These last studies have argued that the increase in joint maximal ROM after stretching training is due to changes in the sensory system (i.e. increase of stretch tolerance) than structural changes in the muscle-tendon unit. However, such studies have conducted the stretching training without accounting for different stretching intensities. Also, the studies were performed for less of 4-weeks, and there are previous studies that suggested that adaptations of the connective tissue occur in a longer time (McClure et al., 1994). Since, the previous studies examining the stretching long-term effects on animal models suggest that the muscle architecture is changed in consequence of the intervention.

Regarding the studies that examined the long-term effects of stretching on muscle fascicles length and angle through ultrasound assessment, no significant changes have been found on muscle architecture (Lima et al., 2014; Nakamura et al., 2012). For instance, Nakamura et al. (2012) performed a 4-week static stretching to the plantarflexors (self-stretching; volume: 2 x 60 s for daily sessions; intensity: "largest stretch that participants were willing to tolerate") and found no changes in fascicle length (FL), fascicle angle (FA), and muscle thickness (MT). Lima et al. (2014) also did not find differences in the MA parameters after an 8-week static stretching program (assisted-stretching; volume: 3 x 30 s with 30 s rest between repetitions, 3 times a week; intensity: "within the physiological limit and preceding the pain threshold"). However, the researchers have suggested that the no changes in muscle architecture could be due to the low stretching intensity and duration (Lima et al., 2014), or simply do not adapt to static stretching training (Nakamura et al., 2012). In addition, there is evidence for higher maximal ROM increase when performing static stretching with a greater stretching intensity (Walter et al., 1996), duration (Matsuo et al., 2013), repetitions (Boyce & Brosky, 2008), and stretching sessions frequency (Marques, Vasconcelos, Cabral, & Sacco, 2009). Thus, it should be examined if a high intensity stretching program would change the muscle architecture.

B – Studies purposes

The present thesis aimed to extent the knowledge about acute and chronic effects induced by stretching with different intensities and durations. Nine studies were conducted for the aim of this thesis (Table 1). The studies 1, 2 and 3 aimed to explore and develop methodological conditions for assessing the joint, muscle and perceptual outcomes to the follow studies. Studies 4, 5 and 6 were designed to analyze the acute mechanical effects on the joint induced by stretching with different intensities and durations. The studies 7 and 8 were conducted to analyze muscle responses during and after stretching with different intensities. The study 9 meant to determine the chronic effects induced a high intensity stretching training on muscle architecture.

Table 1. Organization of studies by research theme and indication of the succeeding studies related.

Issue	Studies number	Studies relation
Assessment methodological considerations	1, 2, 3	2, 4, 5, 6, and 9
Acute effects on joint	4, 5, 6	8
Acute effects on muscle properties	7, 8	
Chronic effects on muscle architecture and maximal ROM	9	

The specific purposes of each study were:

Study 1 – Comparison of different passive knee extension torque-angle assessments [1]

i. Compare two methods (i.e. isokinetic vs. direct measure of resistive torque to stretch and 2D kinematic analysis) to collect passive knee extension torque-angle data;
ii. Analyze the influence of the position of the non-tested thigh on torque-angle outcomes;
iii. Determine the intra- and inter-session reliability of different torque-angle parameters.

Study 2 – A new scale to measure the perception of stretching intensity [2]

iv. Develop an instrument to assess the perception of stretching intensity;
v. Determine the validity and reliability of the stretching intensity scale.

[1] Study published in: **Freitas SR**, Vaz JR, Bruno PM, Valamatos MJ, Mil-Homens P. Comparison of different passive knee extension torque-angle assessments. Physiol Meas. 2013 Nov;34(11):1483-98. doi: 10.1088/0967-3334/34/11/1483. (Impact factor: 1.496)

[2] Work submitted to: **Freitas SR**, Vaz, JR, Gomes L, Silvestre R, Hilário E, Cordeiro N, Carnide F, Mil-homens P. Journal of Strength and Condition Research

Study 3 – Reliability of *In vivo* sonographic biceps femoris (long head) architecture assessment [3]

vi. Develop a still-image (i.e. non-panoramic) ultrasound imagine methodology to assess biceps femoris long head muscle thickness, muscle length, fascicle length and fascicle angle

vii. Determine the reliability of such a technique in resting conditions in vivo using both 3 cm and 6 cm window widths through intra- and inter-examiner assessment;

viii. Determine the smallest detectable change of biceps femoris long head architecture when assessed using ultrasonography.

Study 4 – Responses to static stretching are dependent on stretch intensity and duration [4]

ix. Determine if the stretch intensity and duration have the same impact on human joint passive torque-angle response

x. Determine if stretching for different intensities and equal durations produced different immediate acute effects on passive torque-angle response to stretching.

Study 5 – Are rest intervals between stretching repetitions efficient to acutely increase range of motion? [5]

xi. Determine if non-resting between stretching repetitions provide a higher increase on maximal ROM peak torque, and a decrease of passive torque at a given angle;

xii. 2) Conclude how many repetitions would be necessary to change the maximal ROM, peak torque, and submaximal torque;

Study 6 – Acute stretching effects on the joint passive torque-angle: high-intensity and short-duration vs. low-intensity and long-duration [6]

xiii. Compare if a high intense stretching and low duration would produce similar acute effects on joint mechanical properties compared to a low intensity and long duration stretching

xiv. Characterize the torque-angle timecourse response of a static stretching protocol with high intensity and low duration, and the low intensity and high duration

[3] Work submitted to: **Freitas SR**, Marmeleira J, Valamatos MJ, Blazevich A, Mil-homens P. Ultrasound in Medicine and Biology.
[4] **Freitas SR**, Vaz JR, Bruno PM, Costa P, Mil-homens P. Clinical Physiology and Functional Imaging (in press)
[5] **Freitas SR**, Vaz JR, Bruno PM, Andrade R, Valamatos MJ, Mil-homens. International Journal of Sports Physiology and Performance (in press)
[6] Work submitted to: **Freitas SR**, Vaz JR, Bruno PM, Andrade R, Mil-homens P. International Journal of Sports Medicine

Study 7 – *In vivo* muscle and joint physiological responses to static stretching at different intensities [7]

xv. Examine if the relative decrease in shear elastic modulus is similar at different muscle-tendon length;

xvi. Conclude if the muscle fascicle length is increased in similar ways at the different muscle-tendon lengths during the static stretching;

xvii. Examine if acute effects of stretching are similar in one muscle (i.e., changes in passive muscle tension estimated using elastography) compared to the whole muscle-articular system (i.e., changes in passive torque);

xviii. Determine if stretching decreases the muscle shear elastic modulus at a given muscle length;

xix. Conclude if the effects induced by stretching are intensity dependent;

xx. Observe if the muscle stiffness is changed after static stretching.

Study 8 – Muscle response to a high intensity stretching.[8]

xxi. Determine if the effects induced by a high intensity stretching on passive torque observed in study 6 would also occur for the ankle joint;

xxii. Determine the time course effects of high intensity stretching on muscle passive tension and the maximal isometric force until one hour after the stretching;

xxiii. Conclude if a high intensity stretching would induce muscle damage through maximal isometric force decrease after stretching.

Study 9 – Effect of 8 week high intensity stretching on biceps femoris long head architecture: a pilot study [9]

xxiv. Determine the effects of a 8-week high intensity stretching training program on biceps femoris long head architecture and knee extension maximal ROM;

xxv. Characterize the time course of maximal ROM increase along the 8-week high intensity stretching training program.

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[7] Work being prepared for submission: **Freitas SR**, Andrade R, Larcoupaille L, Mil-homens P, Nordez A.

[8] This study was designed in consequence of the study 6 results. Work being prepared for submission: **Freitas SR**, Andrade R, Mil-homens P, Nordez A.

[9] Work submitted to: **Freitas SR**, and Mil-homens P. Journal of Strength and Condition Research

C – Methods used across studies

In the present section, it is described the methodological aspects similar to all studies. Thus, it is presented the participant's characterization, equipment's and outcomes, and data processing. Specific methodological aspects of the studies are described in the sections of each study, when appropriate.

Participants

A total of 257 participants were involved in the studies (Table 2). The participants of the studies 1, 2, 4, 5, 6, 7, and 9 were all male, in order to eliminate any uncertainty due to gender differences (Hoge et al., 2010; Kubo et al., 2003). The studies 3 and 8 involved both male and female participants. All the participants reported no injuries or orthopedic issues in the lower limbs. The participants of the studies 1, 2, 4, 5, 6, 7, and 9 had a maximum active knee extension lower than 160° (hip was flexed at 90° and 180° of knee corresponds to maximum knee extension).

In respect to the study 2, all participants were Portuguese, physically active, injury-free, and mostly academic students people with an academic degree. In addition, only thirty of the subjects of phase I (age = 21.3±1.9 years, height = 1.76±0.07 meters, body mass = 67.7±8.8kg) participated in the third and fourth sessions (Figure 12, page 20).

Table 2. Demographic characterization of the participants in studies

Study	Group	n	Sex	Age (years)	Height (meters)	Mass (kg)	Tibial length (cm)
1	All	16	Male	21.4±2.1	1.77±0.06	70.6±9.3	37.4±2.4
2	Phase I	60	Male	21.1±1.8	1.77±0.07	71.4±11.8	38.6±4.1
	Phase II	30		26.4±4.6	1.75±0.07	72.4±10.0	36.9±2.7
3	Intra-examiner	20	Male/female	23.0±5.6	1.71±0.11	64.4±12.8	35.5±2.8
	Inter-examiner	10	Male/female	20.8±1.8	1.70±0.13	62.4±14.8	35.5±3.7
4	All	17	Male	23.9±3.6	1.77±0.07	70.5±7.5	37.5±1.6
5	All	47	Male	18.8±3.6	1.75±0.07	71.4±9.1	37.6±2.8
6	All	17	Male	23.9±3.6	1.77±0.07	70.5±7.5	37.5±1.6
7	All	10	Male	27.5±1.4	1.80±0.05	73.9±5.8	-
8	All	11	Male/female	27.2±6.5	1.72±0.10	69.5±10.4	-
9	Stretching	5	Male	21.4±0.5	1.76±0.05	68.5±3.4	-
	Control	4		21.0±1.2	1.76±0.06	78.0±16.6	

This study was conducted in accordance with the declaration of Helsinki and was approved by the local Institutional Ethics Committee (#1/2013).

Equipments and outcomes

Antrophometrical measures

Height, weight, and tibial length were measured using conventional instruments based on the guidelines of the International Society for the Advancement of Kinanthropometry. Tibial length (T_L) was determined as the distance between the medial femoral condyle and the tibial malleolus.

Joint angle

The joint angle was assessed all studies, except for study 3. For the studies 1, 2, 4, 5, 6, and 9, the knee angle was assessed (**Figure 6** and Figure 7), and for studies 7 and 8, the angle was assessed in the ankle (Figure 8). In study 1, the knee angle was assessed using two methods: A) a 2D kinematic analysis coupled to a custom-made device that measured resistance to stretch directly; and method B) an isokinetic dynamometer. For method A, ankle, knee (α_{Knee}), and hip angles were assessed using sagittal-plane digital camera shooting at 50 Hz (JVC, GR-DVL9800U). To determine joint angles, reflective markers were placed over the head of the 1st metatarsal, the medial femoral condyle, and the medial malleolus of the right lower limb; over the greater trochanter of the left femur; and on the left side of the trunk at the intersection of a transverse line passing over the spinous process of the 1st lumbar vertebrae and a line linking the greater trochanter and the midaxillary point (**Figure 6**). The researcher determined smoothing factors using the Ariel Performance Analysis System (APAS®), and the joint angle was then considered for further analyses. For method B, knee angle position was assessed (50 Hz) using the Biodex equipment by aligning the lateral femoral epicondyle with the fulcrum of the dynamometer.

In studies 2, 4, 5, and 6, the knee angle was assessed using the method A of the study 1.

Prior to the tests and while in the starting position of studies 1, 2, 4, 5, and 6, the ankle angles were also measured using a goniometer (Lafayette gollehon extendable, Model 01135). The goniometer fulcrum was aligned with the tibial malleolus, and the goniometer arms were aligned with the 1st metatarsal and the medial condyle of the femur (Figure 7-A).

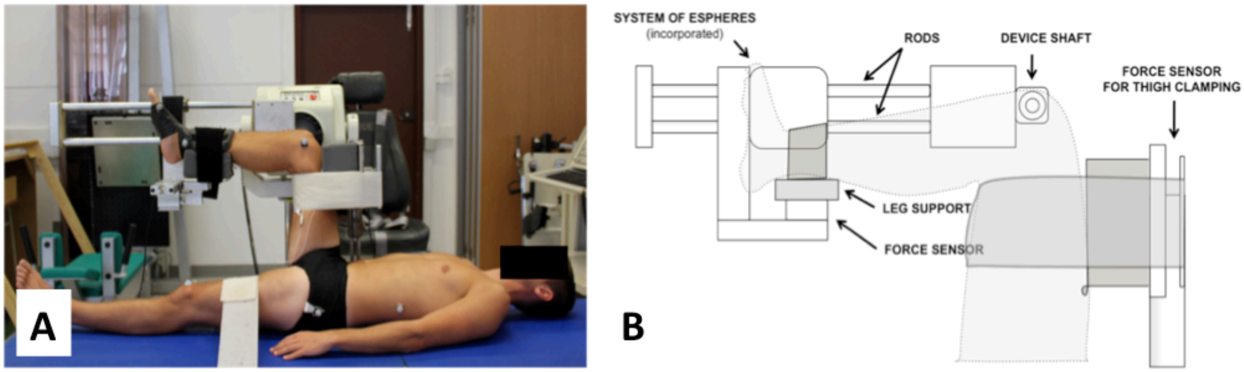

Figure 6. Experimental passive knee extension setup: A) subject in the starting position; B) Schematic representation of the apparatus attached to the dynamometer designed to move the leg with a system of spheres sliding freely in two veins in order to accommodate to lever arm length variations along knee extension

In study 9, the knee angle was assessed using a goniometer (Lafayette gollehon extendable, Model 01135) using the procedure described above (Figure 7-A).

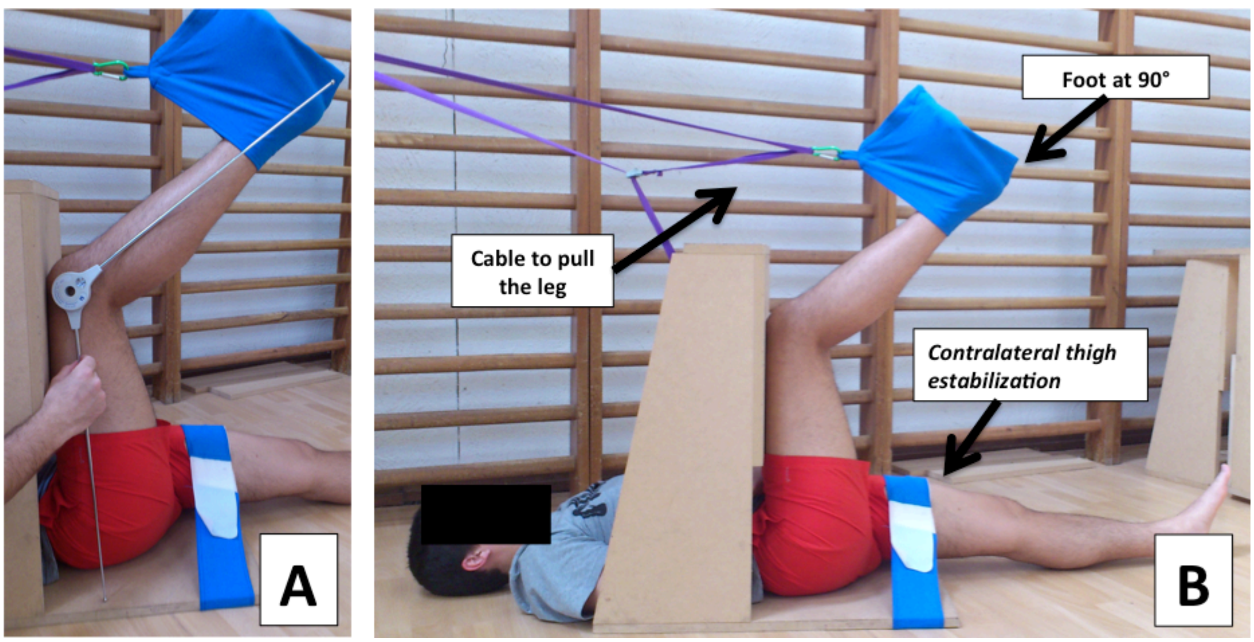

Figure 7. Knee extension goniometric assessment (A) during the stretching procedure of the knee flexors (B) used in the study 10.

For the studies 7 and 8 the ankle angle was assessed using an isokinetic dynamometer (Biodex 3 medical, Shirley, NY, USA), as showed in Figure 8. The lateral malleolus was aligned with the axis of the Biodex. The perpendicular position between the foot and the leg was considered the neutral position (i.e., 0°). Data was recorded at a sampling rate of 1 kHz (MP36, BIOPAC, Goleta, California, USA).

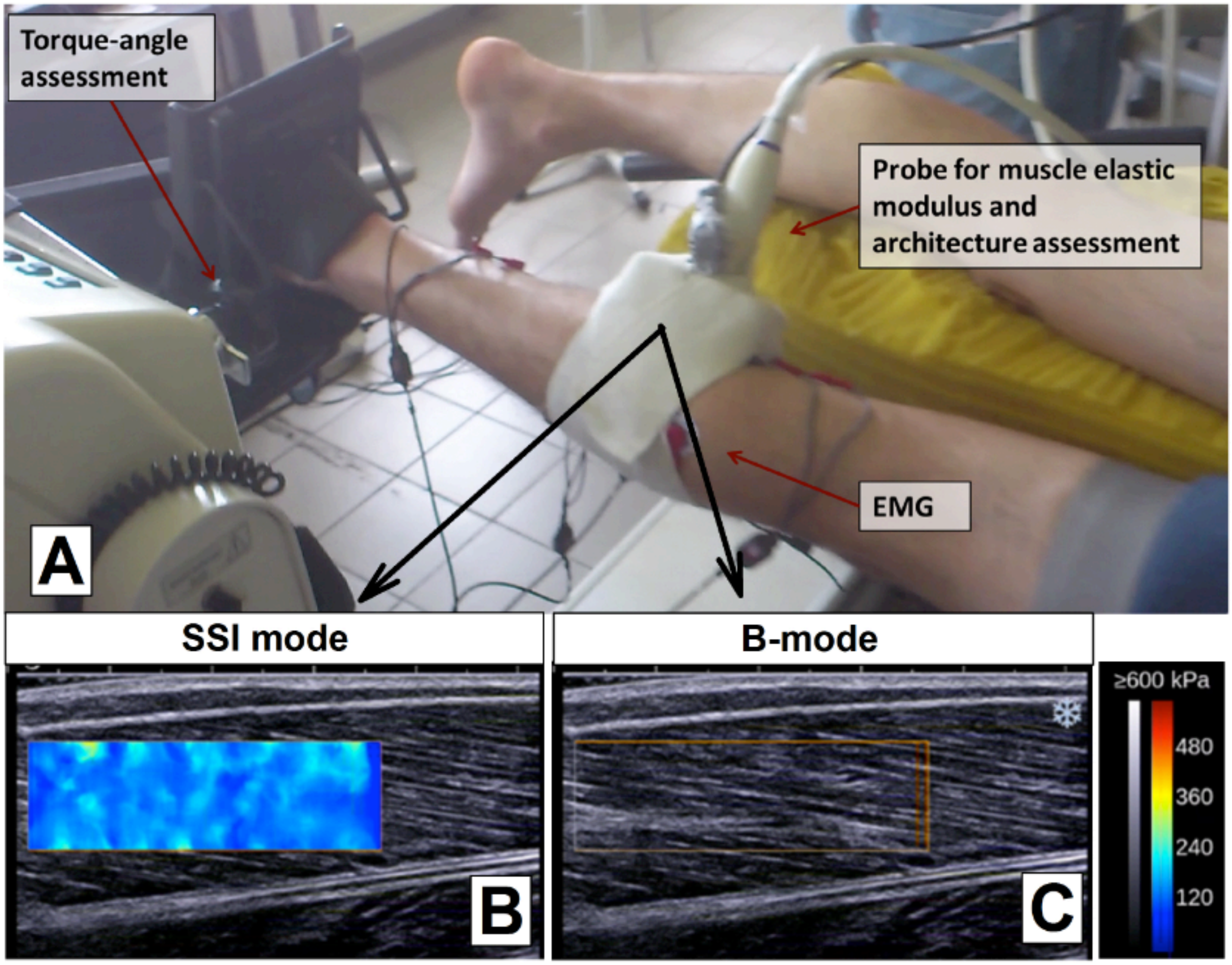

Figure 8. (A) Ankle torque-angle, (B) muscle shear elastic modulus, and (C) muscle architecture assessment setup used in studies 7 and 8.

Passive torque

The joint passive torque was assessed all studies, except for study 3. For the studies 1, 2, 4, 5, and 6, the knee torque was assessed (**Figure 6** and Figure 7), and for studies 7 and 8, the torque was assessed in the ankle (Figure 8).

As the joint angle was assessed in study 1, the knee torque was assessed differently for methods A and B. The equipment described in **Figure 6**-B was designed for method A to measure resistance to passive knee extension force (F_P). It contains a slot for the dynamometer axis and comprised two rods on rollers that slid via a system of spheres and that was connected to the leg support platform. This design enabled free movement of the leg platform relative to the lever device that was moved smoothly by the dynamometer. This setup ensured that the distance from the F_P measurement site to the knee axis was unaffected by a misalignment of the Biodex shaft with the knee axis. A force sensor (platform load cell 1042, Sensor

Techniques Ltd., UK) was incorporated into the leg support platform perpendicular to the subject's leg. For method B, passive torque of the knee was assessed (50 Hz) using the Biodex machine.

In studies 2, 4, 5, and 6, the passive knee extension was assessed using the method A of the study 1.

The Biodex dynamometer was used to impose passive ankle dorsiflexion and to measure ankle torque in studies 7 and 8. Ankle torque-angle data was recorded at a sampling rate of 1 kHz (MP36, BIOPAC, Goleta, California, USA).

Thigh stabilization

The force applied to fast the thigh in the knee extension testing was used in studies 1, 2, 4, 5, and 6 (**Figure 6**-A). A force sensor (platform load cell 1042, Sensor techniques Ltd, UK) was attached to the platform that stabilized the thigh, which enabled measurements (50 Hz) and control of the force that was produced by the Velcro-fixed thigh. Thigh movement was minimized and subject comfort maximized, and the force was determined while setting the subject up for the first test of each study. The same fixation force was used for all tests, and it was recorded throughout testing.

Electromyography

To ensure a completely passive condition during the stretching maneuvers, the muscles activity were assessed in studies 1, 2, 4, 5, 6, and 7 using surface electromyography (EMG).[10] The EMG average amplitude was measured in the semitendinosus (ST) and quadriceps vastus medialis (VM) for studies 1, 2, 4, 5, and 6; and in the medial gastrocnemius (GM), lateral gastrocnemius (GL), soleus (SOL), and tibialis anterior (TA) for the study 7. The electrodes were placed according to SENIAM guidelines (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000), except for the GM muscle. Due to the probe positioning, for this muscle electrodes were placed at the most proximal site of the muscle mid-belly. Surface bipolar electrodes (Plux-Portugal, gain of 1000 for studies 1, 2, 4, 5, and 6; Kendall™ 100 Series Foam Electrodes, Covidien, Massachusetts, USA, for study 7) were placed 20 mm apart (center to center) over the mid-portion of each muscle. The skin, which had been shaved, was roughened slightly using abrasive sandpaper and cleaned with alcohol. The ground electrode was fixed over the left patella for studies 1, 2, 4, 5, and 6; and over specific bony landmarks for the study 8 (Hermens et al., 2000). The EMG signals were amplified (Input Impedance > 100 MΩ; Bandpass Filters=25-500 Hz; CMRR=110 dB) and A/D

[10] In study 8, the EMG was collected, but due to electrical noise from the ultrasound device the data was affected and consequently it is not presented.

converted (MP100 – Biopac™ Systems, 16bits) at a sample rate of 1000 Hz.[11] An examiner regularly monitored EMG during all trials. A trigger was used to synchronize torque-angle and EMG measurements.

Maximal voluntary isometric contraction

The maximal voluntary isometric contraction (MVIC) was determined for knee flexion and extension in the studies 1, 2, 4, 5, and 6; for ankle plantar- and dorsiflexors in studies 7 and 8, for purposes of EMG normalization in all studies, and for maximal strength assessment in study 8. All strength testing was performed using an isokinetic dynamometer (Biodex 3 medical, Shirley, NY, USA). For knee testing, three maximal voluntary isometric knee extensor and knee flexor contractions (MVIC) were performed in the end of the sessions, while in a seated position with the knee and hip at 90° for 5-s contractions using 10-s breaks. For the ankle muscles, three 5-s maximal voluntary plantarflexors and dorsiflexors isometric contractions with 15-s breaks were performed, with the ankle at 0° (i.e. neutral position).

Muscle shear elastic modulus

The medial gastrocnemius shear elastic modulus was measured in studies 7 and 8. An Aixplorer ultrasound scanner (version 7.0; Supersonic Imagine, Aix-en- Provence, France) coupled with a linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France) was used in shear wave elastography mode (musculo-skeletal preset) to assess medial gastrocnemius shear elastic modulus (Figure 8, page 44). The transducer was held statically with a custom-made cast placed perpendicularly to the skin and according the orientation of the muscle fascicles. Assuming a linear elastic behavior, the muscle shear elastic modulus (μ) was calculated as follows (1):

$$\mu = \rho \cdot Vs^2 \quad (1)$$

where ρ is the density of soft tissues (1000 kgm^3) and Vs is the shear wave speed. The probe was positioned over the gastrocnemius muscle mid-belly by an experienced examiner. The maps of the shear elastic modulus were collected at 1 Hz with a spatial resolution of 1 × 1 mm. The timing of each shear elastic modulus measurement was determined using the signal from a microphone (MB Quart K800, frequency response: 40-18000 Hz, sensitivity: 16.25 mV/Pa) recorded using the same device than torque and angle (MP36, BIOPAC, Goleta, California, USA) in studies 7 and 8. For the study 7, during the static

[11] Except for study 1, where the EMG data was collected with a sample rate of 800 Hz.

stretching, the maximal duration of the videos was ≈ 2 min and 40 s, and ≈ 15 s of recording was missing 3 times. For study 8 the duration of the videos were equal to the stretching testing maneuver.

Muscle architecture

The biceps femoris long head (BF) architecture was assessed in studies 3 and 9 (Figure 19, page 83), and the medial gastrocnemius architecture was assessed in studies 7 and 8 (Figure 8-C, 44). For the studies 3 and 9 an ultrasound device (EUB-7500; Hitachi Medical Corporation, Chiyoda-ku, Tokyo, Japan) in B-mode with a 6-cm 10 MHz linear probe was used. For the 7 and 8 studies an Aixplorer ultrasound scanner (version 7.0; Supersonic Imagine, Aix-en- Provence, France) coupled with a linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France) in a B-mode was used.

For shear elastic modulus GM assessment (studies 7 and 8), the probe was placed over the mid-belly of the medial gastrocnemius and strapped with a Velcro (see an GM sonogram example in Figure 8, page 44). The probe was positioned in a manner so the superficial and deep aponeurosis could be visible like the fascicles could be relatively clearly delineated by the echoes from interspaces between them.

For BF assessment, the probe was manually placed over the mid-belly of the BF where the superficial aponeurosis and the fascicles inserting to the mid-aponeurosis could be visible (please read the detail procedure description on the study 3, page 81). In Figure 19 (page 83) it can be seen an example of a BF sonogram.

Visual analog scale score

A 100 mm visual analog scale (VAS) was used to visually score the intensity of the stretching in the studies 1, 2, and 4 (Borg, 1998). The words "no stretch" and "maximum stretch possible" were placed as the left and right anchors. The linear distance from the left anchor to the subject's mark determined the VAS score. The VAS score of each assessment was normalized to the maximal ROM repetition.

Absolut method estimation

The absolute magnitude estimation (AME) method (Stevens, 1957) was used in sessions of phase I of study 2 to determine the numerical scaling within the stretch repetitions performed with different intensities. Participants were instructed to consider that 100 corresponded to "maximal stretching intensity they could perform without feeling pain". Such intensity was previously determined in the first repetition of each session. Participants were informed that they could attribute "any number bellow or above 100, based on the stretching symptom they perceived". The AME score was determined immediately after each stretching repetition.

Perception of stretching intensity

The perception of stretching intensity was assessed in the phase II of study 2 and in the study 9, using the scale developed in the phase I of study 2 (Figure 16, page 76). Participants received a specific and systematic instruction before and during the scale application (see annexe 1. Instruction for SIS administration, page 165).

Body Chart

The body region that subjects felt the stretching symptoms were also registered in the phase I of the study 2. A body map adapted from Boyd et al. (2009) was used. Participants were instructed to point the sites that best represented the stretching perception.

Onset of stretching sensation

The point at which participants of study 2 (in phase I) start feeling the onset of stretching symptoms (OS) during the stretching repetition was assessed using a trigger that was synchronized with others outcomes (Boyd et al, 2009). The participants hold the trigger in the hand and pressed the button when they felt the first symptoms of stretching in all repetitions.

Scale descriptors.

A list of verbal descriptors was used to determine the term that best described the participant's perceived stretch intensity degree in the study 2. The descriptors were written in Portuguese language: *nenhum* (i.e. none), *muito pouco* (i.e. very few), *pouco* (i.e. few), *moderado* (i.e. moderate), *muito* (i.e. high), *quase máximo* (i.e. almost maximal), and *máximo* (i.e. maximal) for the sub-maximal component of the scale; and the terms *máximo* (i.e. maximal), *pouco supra-máximo* (i.e. little supramaximal), *quase supra-máximo* (i.e. almost supramaximal), e *supra-máximo* (i.e. supra-maximal), for the supra-maximal scale component.

Data processing

Joint torque-angle data were synchronized and recorded using the BIOPAC MP100 Acquisition System (Santa Barbara, USA) for studies 1, 2, 4, 5, 6 and 8, and using the BIOPAC MP36 (Goleta, California, USA) for the study 7. In the case of knee angle for study 1 (α_{Knee}), the angle was additionally obtained using a digital camera. To ensure correct synchronization of the knee angle data obtained via kinematics (study 1), a manual trigger was sent to the A/D converter. The data were then synchronized and processed using a specifically designed automatic routine within MATLAB® v12.0 software (The Mathworks Inc., Natick Massachusetts, USA). For study 1, this routine processed data from both the kinematics and force sensor method (A) and Biodex torque and angle outputs (method B) and produced two torque-angle data outputs. For studies 2, 4, 5, 6 and 8, this routine processed the mechanical data using the method A. Briefly, the routine comprised the following steps: 1) torque from both the dynamometer internal sensor and the force sensors from our custom-made device were filtered using a Butterworth second-order low-pass filter (10Hz); 2) knee passive torque (PT) data from method A were calculated by multiplying passive resistance to knee extension (F_p) by the tibial length (T_L); 3) torque data were gravity-corrected by subtracting the leg-foot-device weight (W_{LFD}) from the torque measured using method A or B (McHugh et al. 1992) using the follow equation (1):

$$PT = F_p \times T_L - \cos(\alpha_{Knee}) \times W_{LFD} \times T_L \ , (1)$$

The routine processed the data with the knee angle (α_{Knee}) and torque (T_{Knee}) from the dynamometer output (method A) and that obtained by kinematics and the force sensor (method B) separately. A specifically designed mathematical model 4) was then fitted to the torque-angle raw data for both the dynamic and static phases.[12] Briefly, the dynamic phase was fitted using the following exponential model:

$$f_D(t) = -b_0 \times \left\{ 1 - \exp\left[\ln\left(1 + \frac{A_0}{b_0} \right) \right] \frac{t}{T_1} \right\}, \ 0 \leq t \leq T_1, \quad (2)$$

where b_0 is the parameter to be estimated, T_1 is the last time of the dynamic phase, and A_1 is the true observed ordinate of T_1 (*i.e.,* the true observed peak torque). The dynamic phase of the non-rest intervals repetitions performed in studies 4 and 8 were fitted with an exponential model given by

$$f_{D_i}(t) = A_{0_i} + b_{0_i} \times \left\{ 1 - \exp\left[\ln\left(1 - \frac{A_{1_i} - A_{0_i}}{b_{0_i}} \right) \frac{t - T_{0_i}}{T_{1_i} - T_{0_i}} \right] \right\}, \quad T_{0_i} \leq t \leq T_{1_i}, \quad i = 2, K \ , 5 \qquad (3)$$

where b_{0_i} is a parameter to be estimated, T_{0_i} and T_{1_i} are respectively the first and the last time of the dynamic phase, A_{0_i} and A_{1_i} are respectively the true observed ordinate of T_{0_i} and T_{1_i}, to the repetition i, $i = 1, K \ , 5$. The static phase comprises two different (rapid and slow) components and was fitted with a double exponential model from a combination of two functions such that:

[12] Mathematical models kindly developed by the Professora Paula Marta Bruno.

$$fs(t) = \begin{cases} A_1 - b_1 \times \left[1 - \exp(-\dfrac{t - T_1}{\tau_1}\right], & T < t \leq T_2 \\ A_2 - b_2 \times \left[1 - \exp(-\dfrac{t - T_2}{\tau_2}\right], & t > T_2 \end{cases} \qquad (4)$$

where b_1, b_2, τ_1 and τ_2 are the parameters to be estimated, T_1 is the last time of dynamic phase, T_2 is the last time of rapid component in static phase, and A_i is the true observed ordinate of T_i, i=1, 2. The parameters were estimated using a non-linear least squares method (Levendberg–Marquardt algorithm), minimizing sum of squared errors (SSE).

In respect to the torque-angle data collected during the studies 7 and 8, the passive torque data was gravity corrected by subtracting the weight of the Biodex attachment and the participant's foot for all motion range.

In addition, the automatic routine also normalized the EMG activity during all stretching repetitions to the maximal EMG obtained in MVIC testing. EMG values are reported as a percentage (%) of MVIC.

The SSI recordings from studies 7 and 8 were exported from software (Version 7.0, Supersonic Imagine, Aix en Provence, France) in mp4 video format and sequenced in jpeg image files. All subsequent processing was performed using standardized Matlab scripts (Matlab, Mathworks). Image processing converted the colored map into shear elastic modulus values. For each image, the average value of shear elastic modulus was calculated over a region of interest corresponding to the largest muscular region for medial gastrocnemius (size ≈ 400 mm²). For the study 7, a blinded examiner visually determined the ankle angle corresponding to the muscle slack length based on SSI-angle relationship (Hug, Lacourpaille, Maïsetti, & Nordez, 2013), in order to confirm that stretching was performed beyond the muscle slack length.

In study 8, for torque-angle, the area under the loading curve (E), the area under the unloading curve (ER) and the hysteresis area (ED) were calculated (Nordez et al., 2009). The normalized hysteresis area (DC) was calculated as follows (5):

$$DC = \frac{ED}{E} = \frac{E-ER}{E} \qquad (5)$$

Sonographic images were manually digitized using ImageJ software (NIH, 1.47v, USA) for studies 3, 7, and 9; and using the automatic tracking method proposed by Cronin et al. (2011) for study 7. A trigonometric linear method described elsewhere (Noorkoiv et al., 2010) was used to manually determine fascicle length, fascicle angle, and muscle thickness (Figure 19). Fascicle length (FL) was calculated using the equation: FL=L +(h/sinβ), where L is the observable fascicle length from the mid-muscle aponeurosis (studies 3 and 9) or deep aponeurosis (study 7) to the most visible end-point, h is the distance between the superficial aponeurosis and the fascicle visible distal end-point, and β is the angle between the fascicle (drawn linearly) and the superficial aponeurosis. In each image of studies 3 and 9, three distinct fascicles were tracked and the mean value was calculated and used for statistical tests. For studies 3 and 9, the muscle thickness was measured as the distance between the superficial and the mid-muscle aponeurosis at three points on the image (proximal, middle and distal). The mean of the three thickness measures were used to calculate the overall muscle thickness in studies 3 and 9. The architecture measures were averaged for the three images taken in each session assessment of studies 3 and 9, as a representative participants outcome.

For the stress relaxation data of study 7, only one image was considered when digitized manually for each testing moment: one 3 seconds after achieving the static stretching position, and one other at the end of the stress relaxation. Three examiners digitized two images of each stress relaxation trial. Examiners were instructed to track the same fascicle in the two images. When the fascicle was not fully visible, fascicle length was determined by interpolating the length of the fascicle line crossing the superficial and deep aponeurosis. For study 7, stress relaxation was calculated for torque, SSI, and fascicle length by subtracting the average value of the 5 last seconds at the end of 10-min static stretch to the value 3 seconds after the peak torque (Tian et al., 2010). For the pre and post stretching cycles of study 7, only one fascicle for a video was considered using the automatic tracking routine. An image with the fascicle tracked was saved for the first video, so the same fascicle could be tracked in the two videos. All images were blinded so the examiners could not identify the participants, image session, or testing condition.

Once the EMG data had been recorded and inspected visually, the raw EMG signals were fullwave rectified, and low pass filtered using a Butterworth 4th order and a frequency cut-off of 12 Hz in studies 1, 2, 4, 5, and 6; and the average value of a 100-ms window was considered for statistical analysis. For study 7 the root mean square of EMG signals were measured using a window of 300-ms. The EMG was then normalized to the EMG of the maximum MVIC tests.

The processing for the psicometric data is detailed in study 2 (i.e. section Psychometric data processing, page 71)

Study 1 - Comparison of different passive knee extension torque-angle assessments

Design

An experimental test-retest design was conceived for the purposes of this study (Figure 9-A). Participants came to the laboratory on three separate sessions for performing passive knee extension tests in two position conditions (Figure 9). The passive knee extension torque-angle were assessed simultaneously using two methods: method A) a 2D kinematic analysis coupled to a custom-made device that measured resistance to stretch directly; and method B) an isokinetic dynamometer. The characteristics of the torque and angle assessments of the two methods are detailed in the Equipments and outcomes section (page 42).

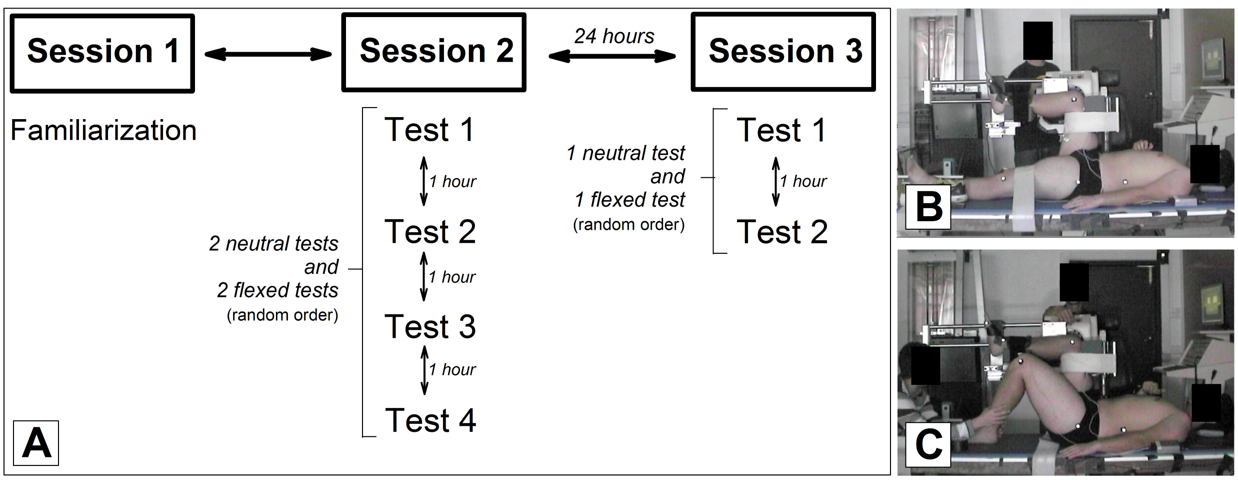

Figure 9. (A) Study 1 design and the passive knee extension torque-angle testing with the contralateral thigh in the neutral position (B) and flexed at 45° (C).

Protocol

In the first session, a familiarization session was performed. Participants performed trials using the passive knee extension setup until they reported confidence with the experimental conditions, and they were then instructed to not perform any strenuous exercise for a period of 24 h prior to the testing sessions. Anthropometric measurements were also obtained during this initial session.

Prior to the second and third sessions, the skin was prepared for surface EMG, reflective markers were placed on specific anatomical reference points, and a functional bandage was applied to the right ankle to immobilize the ankle in a static position. For the second session, the experimental protocol comprised four passive knee extension tests each separated by at least one hour to allow for dissipation of the stretch effects (Magnusson et al., 1995; Ryan et al., 2008). Two repeat tests were performed with the thigh of the

contra-lateral lower limb in a neutral position (Figure 9-B), and the other two repeat tests were conducted with the thigh flexed at 45° relative to the neutral position (Figure 9-C). During the third session, two repeat tests were performed: one with the thigh at 45° and one with the thigh in the neutral position. In total, three measurements were taken for each test condition (45° and neutral): two on the same day and one on a different day. Tests were performed using a balanced order. Inter-session assessments were performed at the same time of day. To mobilize the knee in the dynamic phase, the angular velocity was set to 2°/s, and it was then held for 90-s in the static phase at the maximum knee passive range of motion. Subjects did not perform any type of warm-up or stretching exercise prior to or between experimental tests. Subjects were positioned supine with the right thigh flexed at 90° and fixed to a static platform using a Velcro strap (**Figure 6**). The Velcro was attached to a force sensor that was fixed to the platform that stabilized the thigh (see section *Thigh stabilization*, page 45). The left thigh was stabilized using straps to avoid hip flexion and external rotation and to minimize movement of the pelvic girdle during both position tests (**Figure 6**). To generate passive knee extension measurements, the right leg was strapped firmly to a home-made extension arm that fit to a Biodex system 3 dynamometer (Shirley, NY, USA) (**Figure 6**-B). The right tibial malleolus was aligned to a specific marker on the apparatus during all repeats. All tests began with the apparatus positioned parallel to the ground and the leg at 90° to the thigh.
Subjects were instructed not to move during the testing protocol (in particular to avoid any movement from the trunk and contralateral thigh) and to report the maximum range of knee motion that could be elicited without pain by saying “OK”. An examiner stopped the apparatus upon the subject’s signal and recorded the perception of stretch intensity at the beginning of the static phase on a visual analog scale (VAS).

Maximal EMG activity was determined at the end of each session by performing three maximal voluntary isometric knee extensor and knee flexor contractions (MVIC) while in a seated position with the knee at 90° for 5-s contractions using 10-s breaks.

Statistical analysis

Data were processed using IBM SPSS Statistics 19.0 (IBM Corporation, New York, USA) software. In general, descriptive statistics are reported the mean and standard deviations (mean±SD). However, root mean square errors (RMSEs) are presented as the mean and standard error of the mean (mean±SEM) and residuals are expressed using median and interquartile range (median±IQR). Residuals were determined as the difference between the raw and the fitted torque-angle data for both the dynamic and the static phases. Data were tested for normality using the Shapiro-Wilk test, and no serious violations from normality were noted. A three-way repeated measures ANOVA [method (A vs. B) × protocol (neutral vs. 45°) × measure

(1 vs. 2 vs. 3)] was conducted. The additional assumption of sphericity was assessed using Mauchly's test and was in general confirmed (when it was violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates). Post-hoc analyses were performed using Bonferroni tests. Intraclass correlation coefficients ($ICC_{3,1}$) were chosen based on Chen & Barnhart (2008), and their 95% confidence limits (CI) were computed to determine the reproducibility of the torque-angle parameters between measures. The ICC of torque was determined from a series of 1300 data points or for common sub-maximal knee angles (25, 30, and 35°). The ICCs were grouped as follows: 0.90-0.99, high reliability; 0.80-0.89, good reliability; 0.70-0.79, fair reliability; and <0.70, poor reliability (Currier, 1990). The criterion for statistical significance was set to a p-value < 0.05.

Results

Experimental condition. The conditions during assessments were similar for all tests. No significant differences in ankle angle, hip angle, and thigh-clamping force measurements (pooled results of overall assessments of 49±5°, 98±8°, and 89.2±11.8 N, respectively) were observed. For intra-session assessment of both protocols, the mean time between assessments was 92±31 minutes. The muscular EMG activity was no greater than 3% of MVIC for all repetitions.

Method A vs Method B. Assessment by methods A and B produced two distinctive torque-angle responses (Figure 10 and Table 3). Method A showed a lower knee angular velocity and maximum knee angle. Peak torque was greater when using method A. The RMSE of the torque between methods in a 0-35° range of motion was 1.51±0.03 Nm (representing 9% and 7% of torque at 35° knee angle of methods A and B, respectively). The absolute stress relaxation (SR) amplitude was significantly greater for method A, but not when the SR was normalized to the peak torque.

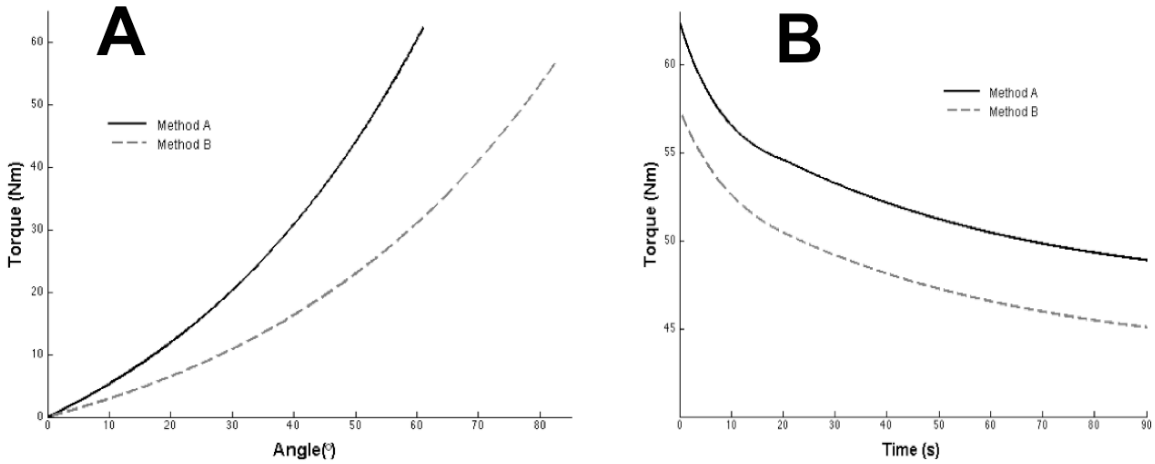

Figure 10. Knee passive extension torque-angle assessed by the two methods in the dynamic (A) and static (B) phases from one subject.

Table 3. Results of torque-angle parameters in assessments by different methods, protocols, and measures.

Method [a]	**A**						**B**					
Protocol [b]	**N**			**45**			**N**			**45**		
Measures [c]	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
AV (°.s^{-1})	1.4±0.1	1.4±0.1	1.4±0.1	1.5±0.1	1.5±0.1	1.4±0.1	1.8±0.1	1.9±0.1	1.8±0.1	1.9±0.1	1.9±0.1	1.9±0.1
MKA (°)	54.3±8.6	54.5±7.2	53.7±7.2	58.6±9.4	58.5±8.4	57.8±6.7	70.6±11.6	71.9±9.2	70.5±8.6	75.1±11.5	76.0±11.2	75.4±8.5
PT (Nm)	50.4±20.0	51.9±22.3	49.2±16.0	43.2±16.9	42.0±13.8	41.5±10.7	44.0±19.0	45.2±20.8	41.4±13.4	37.8±15.8	37.6±13.0	35.6±9.8
AUC (Nm.s)	37619±21008	40106±25173	36381±16069	32585±18092	25334±12062	29212±10489	31432±19010	33619±22561	28823±12894	27683±16114	25334±12062	23711±9018
VAS (mm)	80±9	85±11	78±13	76±14	85±8	80±15	80±9	85±11	78±13	76±14	85±8	80±15
T35 (Nm)	20.5±5.0	22.0±6.5	20.7±5.1	16.5±5.3	16.5±5.0	15.8±4.3	16.9±4.0	18.3±5.9	16.4±4.3	13.8±4.1	13.9±3.8	12.7±3.3
T30 (Nm)	16.1±4.3	17.4±5.6	16.2±4.3	13.0±4.6	12.7±4.2	12.3±3.6	13.0±3.5	14.2±5.1	12.7±3.8	10.7±3.6	10.6±3.2	9.7±2.7
T25 (Nm)	11.8±3.5	12.8±4.6	11.9±3.4	9.5±3.7	9.2±3.4	8.9±2.8	9.4±2.8	10.4±4.1	9.2±3.1	7.7±3.0	7.5±2.5	6.9±2.1
ARS (Nm)	11.3±4.7	11.5±6.5	10.5±3.9	10.8±6.2	10.0±4.3	8.6±3.4	9.7±4.2	9.8±5.1	9.2±3.0	8.8±4.7	8.3±3.4	7.2±2.7
RSR (%)	22.3±4.2	21.7±5.2	21.2±2.8	23.6±6.7	23.1±4.7	20.4±4.8	22.5±3.9	21.9±4.8	22.4±2.9	22.7±4.8	22.2±4.5	20.2±3.9
OR (Nm)	0.14±0.45	0.12±0.50	0.12±0.30	0.24±0.72	0.20±0.47	0.12±0.15	0.25±0.54	0.37±0.59	0.29±0.19	0.60±0.56	0.36±0.68	0.31±0.22
DPR (Nm)	0.30±0.75	0.34±0.81	0.24±0.84	0.54±1.88	0.45±1.47	0.26±0.40	0.48±0.99	0.70±1.13	0.55±0.49	1.11±1.16	0.80±2.07	0.63±0.73
SPR (Nm)	0.05±0.08	0.05±0.11	0.05±0.09	0.11±0.16	0.05±0.08	0.04±0.06	0.13±0.06	0.14±0.08	0.14±0.04	0.17±0.10	0.13±0.64	0.12±0.06

Values are reported as mean±SD, except residuals (OR, DPR, SPR) that are presented as median±IQR. Measures 1 and 2 were taken in the same day and measure 3 in a day apart. Legend: AV - Angular velocity; MKA - Maximum knee angle; PT - Peak torque; AUC - Area under the curve; VAS - Visual analog scale score; T35 - Torque at 35°; T30 - Torque at 30°; T25 - Torque at 25°; AVSR - Absolute stress relaxation; RVSR - Relative stress relaxation; OR - Overall residuals; DPR - Dynamic phase residuals; SPR - Static phase residuals.

[a] - Significant differences were found between methods A and B in all variables, except for OR, DPR, and SPR (*P*< 0.05).

[b] - Significant differences were found between N and 45 protocols in all variables, except for OR, DPR, and SPR (*P*<0 .05).

[c] - No significant differences were found between measures taken in session 1, 2, and 3 in all variables (P> 0.05).

Method A showed lower residuals for the dynamic phase, but not for the static phase. Higher residuals were observed for method A for the initial ~10-s of SR compared with the remaining SR time (0.07 vs. 0.04 Nm) but not for method B (0.14 vs. 0.15 Nm, respectively). However, these differences were not significantly different.

Using a series of 1300 data points, ICCs for 0-35° knee angle range were high for both methods for both intra-session (ICC = .99 and .98, for methods A and B, respectively) and inter-session (ICC = 1.00 and 0.99, for methods A and B, respectively) assessments. For common submaximal knee angles of 25, 30 and 35°, the reliability of torque measurements was good for both intra-session (ICC = 0.80, 0.81, and 0.81, respectively) and inter-session (ICC = 0.84, 0.84, and 0.84, respectively) assessments. The RMSE of torque-angle in the neutral position was 1.7±0.5 Nm for intra-session and 1.6±0.3 Nm for inter-session assessments (representing 3.8% and 3.5% of the peak torque attained within the first trial, respectively).

Methods potential errors. No statistically significant differences between sessions or testing conditions were observed for leg position with respect to device, misalignment between knee and dynamometer axes or distance between markers. The RMSE of the leg position with respect to the device was 0.8±0.5 (intra-) and 0.7±0.6 (inter-session assessment). The RMSE of the distance between the ankle-knee and the knee-hip reflective markers was 0.4±0.3 and 1.2±1.1 for intra-session and 0.8±0.6 and 1.6±1.6 for inter-session assessment, respectively. The misalignment between the knee and dynamometer axes was 5.4±1.6, 5.5±1.5, and 5.3±1.3 for measures 1, 2, and 3, respectively.

Protocol condition. Neutral and 45° protocols resulted in different torque-angle responses (Figure 11-A). Torque for the neutral condition was greater at 25, 30, and 35° (Table 3). However, subjects responded differently to the protocols (Figure 11-B). The neutral condition showed (pooled mean of the three measures) more 19.2% of peak torque, 24.2% of area under the curve, and 7.1% reduction in maximum knee angle. No differences in perception of stretching intensity at maximal angle were observed. A lower absolute SR was observed for the 45° protocol but no differences were found when SR was normalized to peak torque.

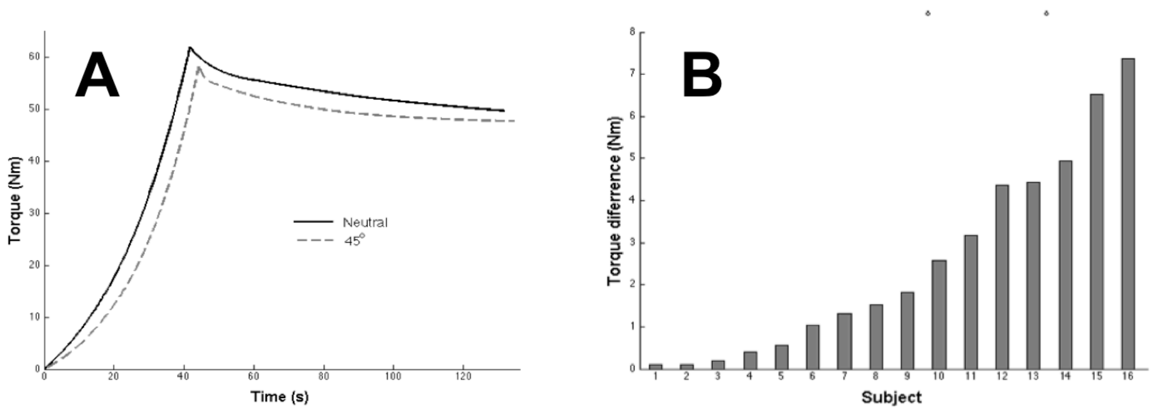

Figure 11. Comparison between neutral and 45° protocols: A) Example of one subject knee passive extension torque-angle response in both protocols; B) Torque difference between protocols for each subject at 30° knee angle (ranked by torque difference).

The mathematical model showed a good fit to the data derived from both protocols and showed a pooled overall RMSE median of 0.15 Nm for both the dynamic and static phases (0.32 Nm and 0.05 Nm in the dynamic and static phases, respectively). For both the dynamic and static phases, no differences in residuals were found between protocols. For the static phase, a higher value of residuals was observed during the initial 10-s (0.07 Nm) when compared with the remaining stress relaxation (0.03 Nm).

Using a 1300-data point series, the reliability of torque for both protocols was high for intra-session (ICC = 0.99 for neutral and ICC=1.00 for 45° protocols) and inter-session (ICC=1.00 for neutral and ICC = .99 for 45° protocols) assessments. Torque reliability data for knee angles of 25, 30 and 35° of both protocols are presented in Table 4. For the neutral protocol, the RMSE of torque-angle test-retest was 2.2±0.4 Nm for intra-session and 1.7±0.3 Nm for inter-session (i.e., 4.3% and 3.4% of peak torque of the first trial, respectively) assessments. For the 45° protocol, the RMSE were 1.5±0.3 Nm and 1.5±0.3 Nm (i.e., 3.5% and 3.4% of the peak torque of the first trial, respectively).

Table 4. Intraclass correlation coefficient (ICC) of torque-angle at 25, 30 and 35° for each protocol by the method A.

Protocol	**Measures**	**ICC (95% CI) of torque**		
		25°	30°	35°
45°	*1 - 2*	0.87 (0.68-0.95)	0.88 (0.69-0.96)	0.88 (0.70-0.96)
	1 - 3	0.87 (0.67-0.95)	0.88 (0.69-0.95)	0.87 (0.69-0.95)
Neutral	*1 - 2*	0.84 (0.58-0.94)	0.84 (0.58-0.94)	0.84 (0.58-0.94)
	1 - 3	0.87 (0.69-0.96)	0.87 (0.67-0.95)	0.87 (0.66-0.95)

Data analyses. The ICC's of the slope of the torque-angle curve and the parameters of the fitted model are presented in Table 5 and Table 6, respectively. The ICC values differed between the type of torque-angle curve outcome and varied based on angle range, the specific angle analyzed, and the type of protocol used. The ICC of the slope of torque-angle curve was greater for larger knee angle range (e.g., 0-35° vs. 30-35°) and was slightly higher for the 45° protocol at knee angles of 25, 30 and 35° (Table 5). The ICC for superior knee angles tended to be lower for both protocols. The ICC of model parameters (Table 4) was poor for b_0 in the 0-35° range (0.48-0.61 for reliability within the same day and 0.39-0.69 for different days) and poor to fair for the torque-angle data where subjects produced a maximum knee angle (.64-.77 within the same day and 0.61-0.82 for different days). With regard to the A_1 parameter, ICC was good to high for both the 0-35° and total torque-angle range.

Table 5. Intraclass correlation coefficient (ICC) of slope of the tangent to the torque-angle curve at 25, 30, 35° and in portions of torque-angle curve corresponding to 0-35°, 20-35°, 30-35°range, for each protocol by the method A.

Protocol	Measures	ICC (95% CI) of slope					
		25°	**30°**	**35°**	**0-35°**	**20-35°**	**30-35°**
45°	*1 - 2*	0.89 (0.72-0.96)	0.87 (0.68-0.95)	0.83 (0.56-0.94)	0.88 (0.70-0.96)	0.88 (0.70-0.96)	0.85 (0.62-0.94)
	1 - 3	0.87 (0.68-0.95)	0.85 (0.61-0.94)	0.78 (0.47-0.92)	0.88 (0.69-0.96)	0.86 (0.64-0.95)	0.81 (0.53-0.93)
Neutral	*1 - 2*	0.83 (0.58-0.94)	0.82 (0.57-0.94)	0.82 (0.56-0.93)	0.84 (0.58-0.94)	0.83 (0.57-0.94)	0.82 (0.56-0.93)
	1 - 3	0.85 (0.62-0.95)	0.83 (0.57-0.94)	0.78 (0.48-0.92)	0.87 (0.66-0.95)	0.84 (0.59-0.94)	0.80 (0.52-0.93)

Table 6. Intraclass correlation coefficient (ICC) of parameters (b_0 and A_1) of the mathematical model fitted to torque-angle corresponding to 0-35° range, and to 0-maximum joint angle (*i.e.* total), for each protocol by the method A.

Protocol	Measures	ICC (95% CI) of parameters			
		0-35° torque-angle		**Total torque-angle**	
		b_0	A_1	b_0	A_1
45°	*1 - 2*	0.61 (0.18-0.81)	0.87 (0.66-0.95)	0.64 (0.25-0.86)	0.91 (0.76-0.96)
	1 - 3	0.39 (-0.55-0.72)	0.87 (0.67-0.95)	0.61 (0.20-084)	0.67 (0.27-0.87)
Neutral	*1 - 2*	0.48 (0.03-0.78)	0.82 (0.54-0.93)	0.77 (0.44-0.91)	0.89 (0.70-0.96)
	1 - 3	0.69 (0.31-0.89)	0.84 (0.59-0.94)	0.82 (0.56-0.93)	0.90 (0.73-0.96)

Discussion

The main findings of this study were: 1) different assessment methods resulted in two distinct torque-angle responses for both the dynamic and static phases of passive knee extension; 2) altering the position of the non-tested thigh affects both the torque-angle response and stretch tolerance; and, 3) different parameters of the same torque-angle data provide different reliability results.

Studies that assess passive joint torque-angle using dynamometry often report it to be a potential source of bias (Magnusson et al. 1995; Hoang et al. 2005; Nordez et al. 2006; Herda et al. 2012). Despite this limitation, dynamometry assessments are widespread because they are less time consuming and require less manual effort and because equipment that can overcome the experimental difficulties associated with passive torque-angle assessment are rare. However, torque-angle response differed between methods of measurement and thus different torque and angle data were obtained. Knee angular velocity was lower when using method A compared with B and lead to different maximal knee angle measurements. This latter issue is related to a misalignment between the joint axis and the dynamometer shaft due to thigh movement during knee extension (that cannot itself be avoided without discomfort to the subject). It can only be attenuated by increasing the fixation of the body segments that are involved in the experiment. However, evidence suggests that when transversal forces are applied to the tissues being stretched, they can affect the torque measured (Rushton & Spencer, 2011). Thus, care must be taken to maintain consistent clamping force across the thigh between repetitions. In our study, no differences were found in the thigh clamping force between repetitions, which may have contributed to the consistent and reliable torque values that we observed. Joint and dynamometer axis misalignment also affected torque measurement because the leg position relative to the lever connected to the dynamometer changed. In addition, methodological errors also justify these varying torque-angle responses. A higher displacement of misalignment between the knee and dynamometer axes (pooled mean RMSE of 5.4±1.5) was observed when compared with the ankle-knee marker distance (0.4±0.3) or leg position relative to the device (0.8±0.5). These results indicate that error from method B (i.e., displacement of knee) impacted torque measurement to a greater extent than errors from method A. Thus, two distinct torque-angle responses were observed.

Moreover, method A also showed a lower residual value between the modeled and the raw data for both the dynamic and static phase compared with method B. We speculate that this difference may be due to the fact that the force sensor was closer to the leg and had less structural degrees of freedom than the dynamometer torque sensor. The residual values that we noted were consistent with results from other studies that tested the ankle (Duong et al., 2001; Hoang et al., 2005), although we found no studies of the knee.

ICC values for both methods were good for intra- and inter-session reliability assessments of torque at submaximal angles. However, the measurements from method B showed lower ICC values than those for method A in the case of both intra- and inter-sessions assessments (0.80-0.81 vs. 0.84-0.88 and 0.84 vs. 0.87-0.88, respectively). This outcome may be due to repositioning of the test subjects between assessments and suggests that measurements performed using method A are more reliable. Additionally, a correlation between passive torque and RMSE between assessments was observed (slope=2.04-2.62; y-intercept=6.41-15.48; Pearson r=0.56-0.83; for both protocols at 25, 30, and 35° knee angles). We assume that errors may be altered depending on the degree of stiffness of the joints. Future experimental studies should consider this possibility.

The absolute and relative RMSE of methods A and B were similar (1.5-2.2 and 3.4-4.3%, respectively) and comparable to previous studies (Hoang et al., 2005). However, surprisingly, the RMSE of testing on different days were somewhat lower than those within the same day (3.8-4.3% vs. 3.4-3.5%). This finding suggests that the method was more reliable when the measurements were performed on different days. This result contradicts the conclusions of previous studies (Magnusson et al., 1995; Hoang et al., 2005) and may be due to pre-trial procedures. For this study, the subjects did not perform any prior stretching maneuvers prior to testing because the aim was to determine reliability in this condition and to replicate in further studies examining acute effects. Other studies have typically incorporated pre-trial stretching exercises for the muscle-tendon units that are involved in the experimental condition to mitigate the effects of time-dependent deformation including thixotropic effects (Magnusson et al., 1996; Hoang et al., 2005; Nordez et al., 2009; Nakamura et al., 2011). This stretching affects the torque-angle response temporarily depending on the type of stretching exercise and the duration of the stretch (Magnusson et al., 1995; Duong et al., 2001; Ryan et al., 2008; Nordez et al., 2009). However, we are unaware of the extent stretching exercises affect reliability when performed prior the experimental protocol.

The second finding of this study was that flexing the non-tested thigh at only 45° caused a lower peak torque and higher knee angle. This difference may be explained by the position of the pelvis (Bohannon et al., 1985) that may have changed because of the connective tissue linking the non-tested lower limb to the pelvis, lower back bones and the tested lower limb (Myers, 2004). However, not all subjects displayed the same order of magnitude of response to thigh position changes (Figure 11-B). This fact may be due to structural, morphological, and body composition differences between the subjects. Moreover, the variation in subject tolerance reflected a different maximum knee angle and peak torque between testing conditions. Thus, despite different torque-angle values for the same joint, the VAS score was similar for both 45 and N protocols. This fact may be explained by a possible change in position of the pelvis or another mechanism triggered by the non-tested limb that may have affected the perception of stretching intensity.

In other studies that assessed knee passive extension, Magnusson's experimental protocol is typical, where the subject is seated in the dynamometer chair and the tested thigh is flexed to 30° to the horizontal plane (Magnusson et al., 1995; Læssøe & Voigt, 2004; Nordez et al., 2006; McHugh et al., 2012; Herda et al., 2012). We do not know if reliability is more affected when in this testing position. Moreover, some studies argue that gains in flexibility are a consequence of stretch tolerance mechanisms (Magnusson *et al.*, 1996). As observed in our study, both stretch intensity and torque-angle outcomes are easily changed by the position of a non-tested body segment. We speculate if the reliability of Magnusson's protocol is not affected, by small changes of the position of body segments. Thus, we suggest a requirement for further studies examining the effects of varying body positions on reliability outcomes.

Finally, our third finding was that torque-angle test-retest reliability varies with the outcome analyzed. Three types of outcomes have been used in previous studies: the torque value for a certain angle or vice-versa; the slope of the tangent, for different ranges of angles, to the torque-angle curve; and the parameters of mathematical models representative of the torque-angle raw data. In our study, we observed that the ICC was consistently superior for torque values at different angles compared with other outcomes. This result is consistent with results from Gombatto and colleagues (Gombatto et al., 2008). In respect to the slope outcomes, the ICC tended to be higher in common range angles compared with specific angles. Nevertheless, for specific angles, the ICC of the slope tended to be higher in inferior degree angles. It tended to be higher for common angle ranges as long the range angle increased. This finding leads to the conclusion that the slope of the superior portion of the torque-angle curve tends to be more consistent, in general, and, as a consequence, results in a higher ICC. However, it should be noted that changes in torque values along the joint range of motion may modify the slope of the torque-angle curve (Herda et al., 2012). Thus, torque-angle slopes depend primarily on torque changes.

As discussed above, the slope of the torque-angle curve has been determined differently in different studies. For instance, McHugh et al. determined slope over an absolute angle range (20-50°) when testing a straight-leg raise (McHugh et al., 1992). Nakamura and colleagues proceeded in a similar manner to these latter authors except that they used a different joint, i.e., the ankle (Nakamura et al., 2011). Magnusson et al. calculated slope using a final third angle range of the torque-angle curve (i.e., linear portion) (Magnusson et al., 1996). Gombatto et al. first determined the quartiles of the total range of motion and then calculated the slope of the torque-angle curve for each quartile in a trunk-bending protocol (Gombatto et al., 2008). Nordez et al. used a representative index of the torque-angle curve shape by calculating the constant of the second derivate of the torque-angle 4th degree polynomial model

(Nordez et al., 2006). Ryan et al. determined the slope of a specific common angle (i.e., second to last common joint angle among subject repetitions) (Ryan et al., 2008). Because the ICC outcomes vary depending on the portion of the torque-angle curve analyzed, interpretation of data and comparison of data between studies is compromised.

With regard to the reproducibility of representative torque-angle modeling parameters, in some studies, it is not explicit whether the mathematical parameters were determined for a given set of torque-angle data from repetitions that were performed until the maximum joint angle that was tolerable to the subject was attained or whether the data pertained to common range of angles that were performed on all subjects (Hoang *et al.*, 2005; Nordez *et al.*, 2010). The results of our study indicate that the ICC of the B0 constant (equation 2) for the common 0-35° angle range was low but was acceptable when determined over the full range of motion despite using the same torque-angle data repetitions. For the A_1 constant, ICC was less affected by specific angles or over a specific range of angles because this outcome was representative of constant peak torque values. Previous studies have used mathematical model parameters of torque-angle data to test the reliability of the assessment in question (Hoang et al., 2005; Nordez et al., 2006). The results of our study indicate that the reliability of mathematical parameters is lower when compared with torque or slope values of torque-angle curve for both within- and between-day assessments. We assume that the results presented in this paper on the ICC reliability outcomes of torque, slope, and mathematical parameters may be extended to other joints subjected to similar protocols. However, future studies should examine this assumption.

In addition, some previous studies have used Person's correlation coefficient as a test-retest reliability outcome of torque angle-assessment (Magnusson et al., 1995; Nordez et al., 2006). However, this statistical parameter may not be the best measure of reliability for torque-angle data because it only measures the strength of linear dependence between two variables and lacks sensitivity to specific data changes. Instead, intraclass correlation coefficients (ICCs) should be used; however, some caution should be taken because an increase in the number of data points tends to increase ICC. As observed in this study, the ICC of complete torque-angle data series (approximately 1300 data points) was very high but the ICC for torque values for specific angles was lower. Thus, ICC should be obtained for specific angles and not all torque-angle data point series.

Nevertheless, importantly, this study has some limitations: 1) the marker of the tested trochanter was assumed to be aligned with the non-tested trochanter and may have affected the exact positioning of hip/thigh tested in the testing sessions; 2) the clamping force used to fix the leg was not controlled; 3) we observed in some individuals that a degree of tibial external rotation during knee extension may have affected force measurements; 4) the weight of the device may have produced transverse forces during knee extension and thus may have affected the measurement of forces perpendicular to the leg; 5) some subjects reported foot numbness, which most likely was due to fixation of the leg and thigh, that may have affected their perception and, consequently, their maximum knee angle; 6) and finally, the system of spheres that we incorporated into our custom-made device may have affected torque measurements made using the dynamometer. With respect to the latter limitation, the main researcher in this study observed a displacement of the leg platform on the rods of no more than 3 cm, and this displacement decreased the distance between the leg platform and dynamometer shaft for all maneuvers. Considering that the torque that was measured by the dynamometer varied from between 0 and 45 Nm in our subjects, a 3-cm displacement to a shorter lever arm may have resulted in a maximum reduction of only 1.35 Nm in the moment arm compared with a constant lever arm. However, this value is considerably smaller than the differences between the values obtained using the dynamometer and method A. Therefore, we conclude that the measurements obtained using the two different methods are undoubtedly distinct.

In conclusion, the results of this study show that: 1) measurements should be performed using method A rather than method B; 2) precautions with and strict control of the position of non-tested body segments should be taken by researchers because they can affect torque-angle outputs; and 3) test-retest reliability outcomes are specific to the variable being analyzed. We suggest that future studies should take these results into consideration to improve the methodology used to assess passive joint torque-angle.

Study 2 – A new tool to assess the perception of stretching intensity

Design

This study has included two phases (Figure 12). The first phase aimed to develop the scale (i.e., determine the scale's visual, numerical and descriptive components) and to systematize the scale instruction to be used in phase II. The second phase was designed to validate the scale for different items (i.e., content, construct, face, and criterion), test the scale properties' reliability (i.e., stretch intensity prediction and production), determine intra- and inter-tester reliability, and test the scale's responsiveness to acute changes induced by stretching. Four sessions were performed in phase I, and three in phase II. The primary investigator of the study was not involved in data collection, so that the scale administration could be blinded. Thus, three experienced researchers were instructed and trained in the study's procedures, equipment measurement, administration of the scales and questionnaires.

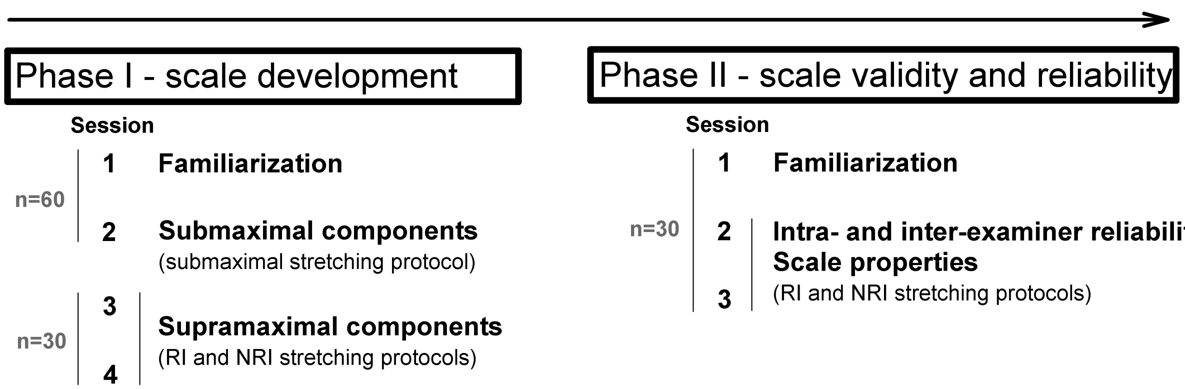

Figure 12. Design of the study 2.

Protocol

Demographic and anthropometric data were collected in the first session for all participants of each phase (Figure 12). Familiarization with the experimental setup and instruction in the protocol procedures were provided before testing. Familiarization involved an explanation of the torque-angle equipment, study protocol, basic concepts of stretching definition and symptoms, the generic body region that participants would theoretically feel stretch, and how to respond to the questionnaires and scales used in this study. Before testing, participants were also asked to rank items in a list of verbal descriptors in an ascending order to ensure recognition of the words meaning. Several procedures were taken until the participants reported confidence and understanding about the protocol of the upcoming sessions. Visual inspection was done in muscle EMG during the passive knee extension testing to ensure that no artifacts were created by emotional or contextual factors. For phase I, one maximal ROM (mROM) without pain repetition was initially performed followed by six submaximal repetitions in a balanced order. Three of these repetitions were determined as a percentage of maximal tolerable torque, from a starting position (SP) to mROM range; and the other three repetitions from an OS to mROM range. The sub-maximal intensities were calculated at 40%, 60% and 80% of the maximal tolerable torque in both ranges. A different frame of reference (SP vs. OS) was used to test which range would have a higher correlation with the physiological responses (i.e., ROM and torque). The submaximal stretching repetitions of OS-mROM (RO40, RO60, and RO80) and SP-mROM (R40, R60, R80) ranges were determined after the initial repetition to mROM and performed in a random balanced order. An examiner stopped the dynamometer on the target submaximal ROM and at mROM for ≈3 seconds before the limb was returned to the SP. Five minutes were given before performing the submaximal repetitions so that the percentage of submaximal intensities could be determined, and to ensure the dissipation of stretching effects (Mizuno et al., 2013; Nordez, McNair, Casari, & Cornu, 2010). To determine the OS-mROM range, the participants were instructed to indicate "the moment [they] feel the first OS" by pressing a trigger that they held during the stretching maneuvers, and to say "OK" when obtaining the "maximum range of motion without pain" (mROM). This procedure was replicated in all stretching repetitions, in order to confirm OS detection reliability. Two-minute rests were given between each submaximal stretching repetition. During this interval time, participants were asked to report stretch symptom body location, to classify the stretch intensity (using stretch VAS score and AME), and to indicate the scale anchor from a list of words that best described its stretch intensity.

In the third and fourth sessions of phase I, two stretching protocols were performed with a balanced order: a rest- (RI) and a non-rest interval (NRI) stretching protocol (Figure 13). It was previously observed that these protocols induce distinct stretching supramaximal intensities (i.e., above maximal ROM of first repetition) among repetitions (see study 5, page 101). Protocols were applied at the same time of day with a one-week interval between the two sessions. Participants were briefly instructed in the protocol procedures at the start of the second session. The protocols consisted of five stretching repetitions with a 30-s rest interval until their mROM without pain (RI); and a maximal number of repetitions without rest interval between repetitions (NRI). Each stretching repetition lasted 90 s in the static phase. The maximal number of NRI repetitions was determined when subjects reported that they could not produce a further repetition without feeling pain (Figure 13). For all repetitions, an examiner stopped the apparatus upon the subject's signaling of mROM. Stretching repetition tests started from the resting position, and the VAS scale and AME method were applied in beginning of the static phase of each repetition. At the end of each session, a pain VAS scale was applied to determine the degree of pain felt during the stretching protocol. With the results of phase I, the stretching intensity scale (Figure 16) was constructed (please see *scale construction* section).

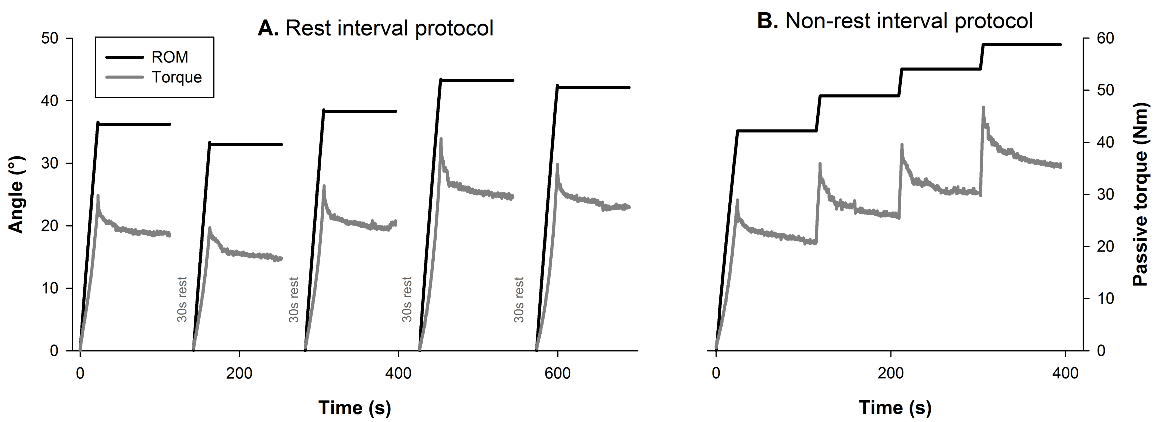

Figure 13. Example for one participant of (A) rest interval and (B) non-rest interval stretching protocols. In this example, the participant has performed five repetitions with a rest interval, and four stretching repetitions without a rest interval.

In sessions of phase II, the two stretching protocols previously used (i.e., NRI and RI) were performed in a balanced order to determine the scale validity and reliability. Before and after each protocol, four repetitions were performed to a target stretch intensity. Four repetitions were used in order to have a minimal number of data points to establish a statistical correlation. The first repetition was performed until the mROM without feeling pain, and the remaining three repetitions were completed in a balanced order by either producing a stretch intensity or estimating the stretch intensity. Half of the participants (n=15) produced the stretch intensities for a stretching intensity scale (SIS) score of 80, 60, and 40. The other half estimated the SIS score at 80% (R80), 60% (R60) and 40% (R40) of the maximal tolerable torque that was previously determined in the first repetition. The same set of repetitions was applied after the protocol in the same order. The dynamometer was held at the target stretching intensity for 3 seconds in the pre and post testing repetitions before the limb was returned to the starting position. The scale was applied immediately after achieving the target stretch intensity of each repetition. In order to determine intra- and inter-examiner assessment reliability, half of the participants (n=15) were assessed by the same examiner in the two stretching sessions, and other half by two different examiners.

At the end of all sessions, participants performed three maximal voluntary isometric muscle contraction (MVIC) repetitions of 5-s duration with a 10-s break for both knee extension and flexion, with the knee at 90°, for the purposes of EMG signal normalization.

Conceptual scale develop, validation and reliability

The SIS was developed to have three components: a visual, a numerical, and a descriptive one (Figure 16, page 76). The visual component was determined based on the VAS score. The numerical component was determined based on the AME score. The descriptive component was determined based on the verbal descriptors chosen from the list by the participants during the stretching trials. Scale construction and validation was based on the concept of Borg's continuum model (Borg, 1998). According to Borg's model, physiological responses should be observed during physical performance. Thus, the joint passive torque and angle were chosen as the stretching physiological outcomes. The study design as well as the content, construct, face, and criterion validity were determined by a stretching specialist professional and an experienced specialist in scale development.

Content validity was determined based on previous studies and participants' understanding (Borg, 1998; Boyd et a., 2009; Freitas et al., 2013). The words used to qualify the nature of the symptoms during the stretching were borrowed from Boyd et al. (2009), who found in a straight leg raise test that the terms used by individuals were essentially "stretching" and "tension". Consequently, we used the term "stretching" to refer to symptoms felt during the exercise. The descriptors used to characterize the degree of stretching intensity were chosen based on words from other scales (Borg, 1998; Ferreira-Valente, Pais-Ribeiro, & Jensen, 2011) translated and adapted to the Portuguese language. The participants' understanding of the ranking order of the sub- and supramaximal terms was tested by asking subjects to sort a group of terms in order of increasing intensity. The metric chosen for the scale using the AME method ranged from 0 to values above 100. The number 100 was considered as referring to mROM without pain. Such metric range was chosen because it was previously observed in a pilot test (not published) to have a comparable concordance with physiological responses as relative (i.e., normalized to maximum values) joint passive torque, angle, and area under the torque-angle curve. The values and position of the scale numbers were based on the VAS score and AME phase I results for both submaximal (i.e., below the maximal ROM) and supramaximal intensities. The VAS has been shown to be a reliable instrument to determine supramaximal measures (Price, 1994, cited by Borg 1998, pp. 24). Construct validity was based on the assumption that the perception of stretch intensity varies with tissue deformation by changing the joint angle, which in turn induces a change in joint passive torque. The SIS criterion validity was determined by ensuring four conditions: 1) that the SIS score would be related to the physiological measures of joint passive torque and angle (i.e., concurrent validity), for submaximal (in both OS-mROM and SP-mROM ranges) and supramaximal intensities; 2) that the SIS would be reliable to predict the ROM and torque (i.e., predictive validity) (Borg, 1998; Coquart, Garcin, Gaynor, Tourny-Chollet, & Eston, 2014); 3) that the SIS would be reliable to produce a certain submaximal stretch intensity of passive torque and ROM (i.e., productive validity) (Borg, 1998; Coquart et al., 2014); and, 4) that the acute effects induced by stretching on ROM and passive torque could be detectable using the SIS score.

Psychometric data processing

Phase I data was used for scale development. All the submaximal repetitions were first ordered according to the ROM performed. After checking the normal data distribution, median values of AME and relative values of VAS score (i.e., normalized to R100 value) were calculated for each submaximal intensity. Then, repetitions with a within-AME score difference of less than 15% were excluded (Portney & Walkins, 2009), except for R100. The remaining repetitions were adjusted to an exponential function (6):

$$y = a \times e^{bX} \qquad (6)$$

The γ is the relative AME score, the a and b are mathematical parameters, and χ is the VAS score. After modeling for Equation 1, the values of relative VAS score (i.e., scale number positioning) for the AME percentiles values from a to 100 were determined. After confirming the descriptors' order initially set by the participants, the most frequent descriptors chosen for each stretch intensity were determined. When a similar descriptor was observed for two successive stretch intensities, the average relative VAS score value was determined for that descriptor. Descriptors that did not follow the initial order set by the participants were excluded. Consequently, the percentile numbers (i.e., AME values) and stretch intensity anchors (i.e., descriptors) were positioned based on their respective relative VAS score values in a 100-mm vertical line (Figure 16, page 20).

In respect to the supramaximal data, all repetitions of RI protocol and the number of maximal NRI repetitions performed by each participant were used for data analysis. After checking normal distribution, the median values of relative VAS and AME score were determined for every repetition in both stretching protocols. The most frequent descriptor was determined for each stretching intensity. Then, all repetitions of both protocols were ordered according the maximal ROM performed. Repetitions with a within-AME difference of less than 15% (Portney & Walkins, 2009) and with a different order than the initial order set by the participants were excluded. The average relative VAS score value was determined when a similar descriptor was observed for two successive stretch intensities. Data was then fitted to a linear mathematical function (7):

$$y = mx + b \qquad (7)$$

The γ is the relative AME score, the m and b are mathematical parameters, and χ is the VAS score. Based on Equation 2 model fitting results, the VAS score values (i.e., number positioning) were determined for every 10 AME score points from the 100 AME value to the maximal AME observed (i.e., 150). Consequently, the AME numbers (i.e., median AME values) and stretch intensity anchors (i.e., descriptors) were positioned based on their respective relative VAS score values in a vertical line above the submaximal scale line (Figure 16, page 20).

Statistical analysis

All data were analyzed using IBM SPSS Statistics 19.0 (IBM Corporation, New York, USA). Normal distribution was confirmed using Shapiro-wilk test. Correlations between physiological (i.e., torque and ROM) and SIS score for both submaximal and supramaximal intensities were determined using Pearson coefficient (r) and intraclass coefficient correlation (ICC) at a 95% confident interval. When normality was not observed, the Spearman's rank correlation coefficient (*rho*) was used. The ICCs were classified as follows: "little" (0.00–0.25), "low" (0.26–0.49), "moderate" (0.50–0.69), "high" (0.70–0.89), and "very high" (0.90–1.00) (Kwah et al., 2013). Intra- and inter-examiner assessment reliability, OS detection reliability, and scale properties (i.e., production and estimation) reliability were determined using r and ICC. The pre-post stretch effects on ROM and torque for scale estimation assessment, and SIS score for scale production assessment, were determined using paired t-tests. The effect size for the changes induced by stretching was determined for the difference using the Cohen's d. Statistical significance was set at $p<0.05$.

Results

Phase I – Scale construction. The EMG activity was below 4% of MVIC in all repetitions of all sessions. All participants ordered all submaximal and supramaximal descriptors in an ascending manner. The body regions reported to be stretched in session two of phase I were the posterior thigh (93.6%), posterior leg (7.6%), and the anterior thigh (3.4%). The average values of pain VAS score in supramaximal stretching protocols of phase I were below 20 mm (NRI=9.7±15.0 mm and RI= 4.7±7.7 mm, $p<0.001$), thus indicating that no pain was felt during their stretching. The detection of OS was found to have a very high reliability (r=0.96 ICC=0.93 [0.87-0.97]). A normal distribution was not found for the AME score, and thus median values were used for all submaximal repetitions. A very high correlation was obtained between the physical (i.e., ROM) and physiological (i.e., knee passive torque) outcomes and the perceptual variables (i.e., relative VAS mean score and for AME median score) in submaximal intensities of both SP-mROM and OS-mROM ranges (Figure 14-A to D). Due to the 15% AME within-differences criterion, the RO80, RO60, and R60 repetitions were excluded, and remaining repetitions were fitted to Equation 1 (Figure 14-E). The equation 6 parameters obtained were a=14.829 and b=0.0189 (r^2=0.98). Consequently, the relative VAS score values (i.e., numbers positioning) were determined for the percentile numbers ranging from 20 to 100 (Figure 14-E).

In respect to the submaximal descriptors, the same term was observed for R80 and RO80 repetitions (i.e., "almost maximal"). Thus, the relative VAS score average value was determined for that descriptor (i.e., descriptor positioning). The descriptor "much" was also excluded, because it did not follow the initial order set by the participants. The most common descriptors for each stretching intensity can be observed in Figure 14-E. The resultant submaximal scale component that was constructed can be observed in Figure 16 (page 20).

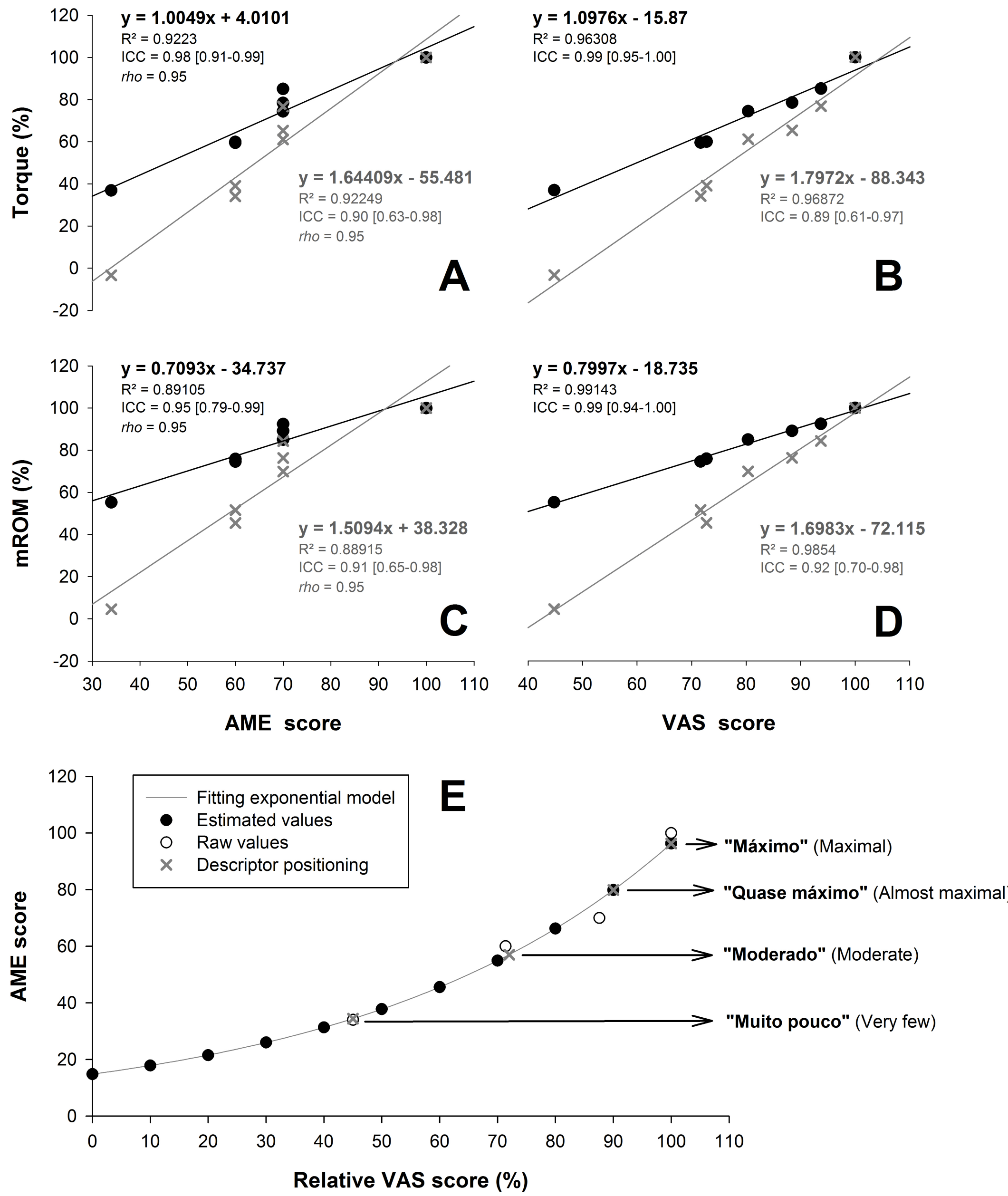

Figure 14. Correlations between relative (normalized to maximal range of motion values) values of: (A) VAS-peak torque, (C) VAS-maximal range of motion (mROM), (B) AME-peak torque, (D) AME-mROM, and (E) VAS-AME for values obtained in the submaximal stretching repetitions. In the graphs A to D scatters are plotted for both SP-mROM (•) and OS-mROM (×) ranges. The VAS-AME data was fitted to an exponential model and the VAS score was estimated for the percentiles of AME (E). The most common descriptors for different intensities are shown in figure E.

For supramaximal data, no normal distribution was observed for the relative VAS score or AME score. Participants performed different numbers of maximal NRI repetitions (2R, n=3; 3R, n=12; 4R, n=10; 5R, n=5). No differences were observed between protocols in the first repetition in maximal ROM or peak torque (NRI=49.2±15.0 vs RI=46.3±13.8, $p=0.20$). A very high correlation was obtained between the physical (i.e., ROM) and physiological (i.e., knee passive torque) outcomes and the perceptual variables (i.e., relative VAS mean score and AME score) and torque in submaximal intensities (Figure 15-A to D). The relative VAS score and AME score fitted to the Equation 7 produced parameter values of m=0.7667 and b=25.751 (Figure 15-E). The estimated relative VAS score for every 10 AME score points from 100 to 150 (i.e., maximal median AME value observed) is shown in Figure 15-E). The fifth repetition of NRI protocol was excluded from the supramaximal scale data analysis, because only two participants performed 2NRI repetitions. After ordering all repetitions according maximal ROM, and using the 15% within-differences criterion, the repetitions R3 and R5 of RI protocol and R4 of the NRI protocol were considered for the descriptors' positioning. Consequently, the supramaximal scale component was designed (Figure 16).

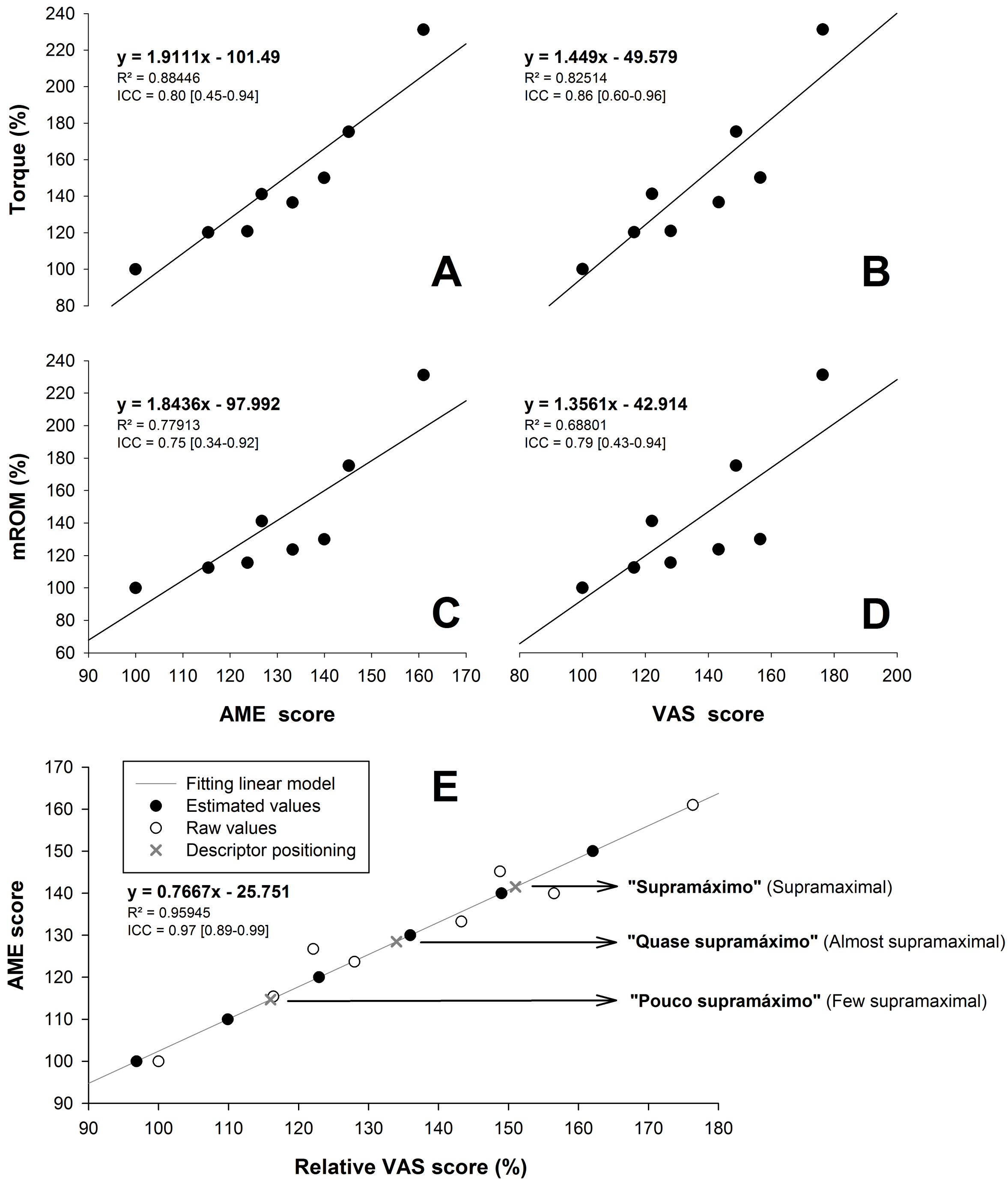

Figure 15. Correlations between relative (normalized to maximal range of motion values) values of (A) VAS-peak torque, (C) VAS-maximal range of motion (mROM), (B) AME-peak torque, (D) AME-mROM, (E) and VAS-AME for supramaximal values obtained in rest interval and non-rest interval stretching protocols. The VAS-AME data were fitted to a linear model, and the VAS score was estimated for every 10 AME points above 100 until 150. The most common descriptors for different intensities are shown in Figure E.

Phase II – Scale validation

The scale constructed in phase I and used in phase II for measuring the perception of stretching intensity is depicted in Figure 16.

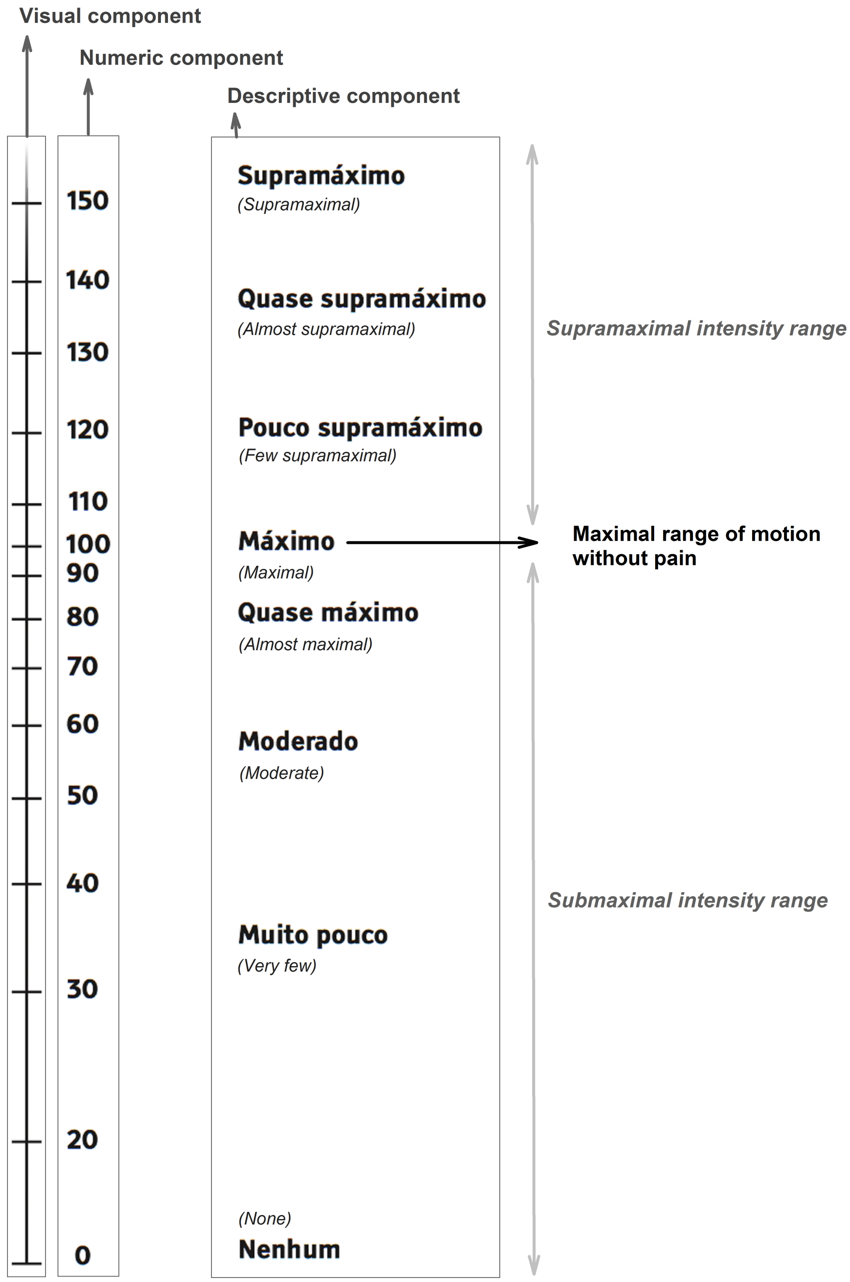

Figure 16. Stretch intensity scale, composed by two intensity dimensions (sub- and supramaximal). The number 100 represents the maximal range of motion without pain. The font used for the lettering and numbering was Tiresias Signfont Regular.

Participants performed different numbers of maximal NRI repetitions (2R, n=3; 3R, n=12; 4R, n=10; 5R, n=5). No differences were observed between protocols in the first repetition in maximal ROM or peak torque (NRI=43.2±9.2 vs. RI=45.0±9.0, p=0.09). The normalized maximal ROM, peak torque and SIS score were 106.2±10.2%, 108.9±21.7%, and 107.4±18.9 for the rest interval stretching protocol, respectively; and were 116.8±14.8%, 128.5±37.6%, and 119.1±19.0 for the non-rest interval protocol, respectively. A typical example of one participant's SIS, torque and angle response before, during, and after two stretching protocols can be observed in Figure 17.

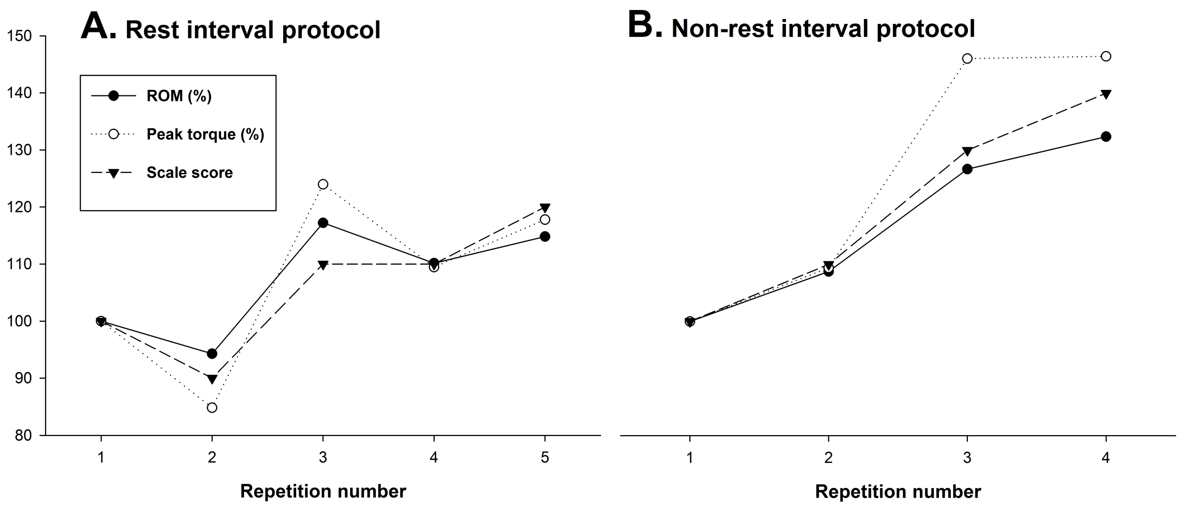

Figure 17. An example for one participant's maximal range of motion (mROM), peak torque, and stretching intensity scale score (A) in rest-interval and (B) non-rest-interval stretching protocols. Peak torque and ROM are normalized to the value of the first repetition. This participant performed four non-rest-interval stretching repetitions.

A moderate to high correlation was found between SIS score and torque (r = 0.88, ICC = 0.85 [0.81-0.88] and r = 0.62, ICC = 0.57 [0.47-0.65] for submaximal and supramaximal intensities, respectively) and between SIS score and ROM (r = 0.85, ICC = 0.85 [0.81-0.88] and r = 0.76, ICC = 0.70 [0.63-0.76] for submaximal and supramaximal intensities, respectively). The reliability for both intra- and inter-examiner assessment, and for the scale production and estimation properties at different submaximal angles is shown in **Table 7**. For all angles, intra- (r=0.88, ICC=0.88 [0.79-0.92]) and inter-examiner (r=0.93, ICC=0.93 [0.88-0.96]) assessment showed high to very high reliability results. Scale estimation property showed an inferior reliability result (r=0.70, ICC=0.70 [0.50-0.83], for all angles) as compared to a scale production property (r=0.89, ICC=0.89 [0.82-0.93], for all angles).

Table 7. Reliability outcomes for intra- and inter-examiner assessment, and for stretching intensity scale properties of estimation and production for repetitions at 40% (R40), 60% (R60), 80% (R80), and 100% (R100) of maximal tolerable torque.

	R40		R60		R80		R100	
	r	ICC (CI95%)	*r*	ICC (CI95%)	*r*	ICC (CI95%)	*r*	ICC (CI95%)
Intra-examiner	0.69	0.63 (0.19-0.86)	0.92	0.92 (0.78-0.98)	0.90	0.89 (0.71-0.96)	0.99	0.99 (0.97-0.99)
Inter-examiner	0.89	0.88 (0.68-0.96)	0.95	0.95 (0.86-0.98)	0.93	0.93 (0.80-0.98)	0.98	0.98 (0.95-0.99)
Estimation	0.06	0.06 (-0.47-0.56)	0.47	0.47 (-0.06-0.79)	0.41	0.36 (-0.19-0.74)	-	-
Production	0.81	0.81 (0.52-0.93)	0.77	0.78 (0.45-0.92)	0.82	0.81 (0.52-0.93)	0.75	0.74 (0.38-0.90)

The pre and post measurements of both production (torque and ROM) and estimation (SIS score) outputs are shown in Table 8.

Table 8. Values of range of motion (ROM) and torque when using the estimation scale method, and stretching intensity scale score (SIS) when using the production scale method, before and after the stretching intervention.

			Pre	*Post*	*p*	*d*
Production	**ROM (°)**	**R40**	15.8±10.2	18.2±11.4	0.001	0,23
		R60	21.5±11.7	24.3±11.9	0.003	0,23
		R80	28.5±14.2	31.5±14.0	0.004	0,21
		All angles	22.0±13.1	24.7±13.5	<0.001	0,20
	Torque (Nm)	**R40**	9.9±5.8	11.3±6.6	0.01	0,24
		R60	15.4±9.3	17.0±8.9	0.03	0,18
		R80	22.8±13.7	25.6±13.6	0.01	0,21
		All angles	16.0±11.3	18.0±11.6	<0.001	0,17
Estimation	**SIS**	**R40**	47.2±11.6	44.0±16.6	0.19	0,03
		R60	67.0±9.1	62.7±16.0	0.04	-0,33
		R80	77.2±11.6	77.7±18.5	0.77	-0,21
		All angles	63.5±16.5	60.6±21.5	0.03	-0,16

Legend: *p* – p-value; *d* – Cohen's *d*

Discussion

In this study, a new instrument to assess the perception of stretching intensity was developed, and was shown to be valid and reliable during a slow passive knee extension maneuver. The results found in the present study support Borg's (1998) findings. Borg's theory assumes that the ratio scales of perceived exertion were based on the assumption that the exertion perceived during physical exercise is stimulated by several biological systems, and consequently that diverse physiological parameters correlate with perceived exertion. The SIS score was accompanied by changes in joint ROM and passive torque in both submaximal and supramaximal stretching intensities with a surprisingly high sensibility, when compared to the validity results from other studies (Borg, 1998). Joint passive torque is mainly changed by tissue deformation (either tension or compression), and joint angle affects tissue length. Changes in both variables affect afferent drive to the central nervous system, thereby mediating the pain pathways (Holdcroft & Jaggar, 2005). In this study, for the purpose of concurrently validating the SIS, the joint passive torque was chosen instead of the muscle-tendon tension measurement. This was chosen because it was conceptualized that stretching perception would be generated as a result of the net forces caused by the deformation of the tissues crossing the joint, and not a single tissue; however, it remains unknown whether a higher correlation could be obtained to a direct measure of a specific tissue's passive tension. The passive tension of tissues could be assessed using supersonic shear wave imaging elastography *in vivo* during a stretching maneuver (Maïsetti et al., 2012). Thus, future studies should extend this validation by correlating the SIS score with the passive tension of tissues.

The maximal reference was taken in this study as the maximal ROM without pain. Such criterion is often used in clinical and sports contexts (Behm & Kibele, 2007; Boyd et al., 2009; Walter et al., 1996). Based on this reference, participants scored the stretching intensity perception on SIS, for both submaximal and supramaximal intensities. Thus, we strongly suggest in future applications of SIS that when instructing the participants for SIS usage, emphasis should be placed on the fact that SIS scoring must be performed in reference to the mROM without pain. This procedure increases the reliability of the scale, as was demonstrated in this study for both intra- and inter-examiner assessment. In addition, to ensure that mROM is well understood and memorized by the participants, we suggest that the examiners when determining the mROM should ask in an understandable way whether participants believe that they achieved mROM; also, if needed, the participants should repeat the trial. This is an important procedure, because the SIS scoring will depend on the accuracy of this reference.

The validity of SIS was also confirmed by the scale properties to produce or to estimate a certain stretching intensity (Borg, 1998; Coquart et al., 2014). We found a high reliability in scale production but a low to moderate reliability for scale estimation in different submaximal intensities. When analyzing for all intensities tested, both SIS production and estimation methods showed high reliability. This suggests that SIS is better for producing a certain stretching intensity based on SIS than reporting the SIS score in certain intensity. Because no previous studies have assessed stretching intensity using an estimation method, we are unable to compare these results; however, the results obtained for different individual submaximal intensities were very low. We assume that with longer familiarization and training, the SIS estimation method reliability might improve (Borg, 1998). Thus, we suggest that future studies explore this property of the scale, since there are few studies comparing the estimation and production methods.

A specific characteristic of SIS is the supramaximal component, which allows the detection of stretch intensity above the maximal ROM with a high sensitivity. It is known that ROM increases among stretching repetitions, and there are methodological procedures that facilitate the increase of stretch intensity (see study 5; Sharman et al., 2006). However, when performing stretching training, it is difficult to assess the magnitude of these changes. In the present study, the participants performed two different stretching protocols that led to two distinct levels of stretch intensity. This means that the RI protocol produced a smaller increase in torque and ROM among repetitions than the NRI stretching method; however, although different changes of ROM and torque were produced by the two protocols, the SIS proved capable of discriminating and following up on these changes (Figure 17). Thus, a high correlation between the SIS score and the physiological changes was observed. This means that SIS can be used to detect changes in ROM and peak torque above the maximal ROM when performing stretching training.

The SIS was also shown to be capable of detecting acute torque-angle changes induced by stretching. It is known that stretching induces an acute decrease in joint passive torque and an increase of maximal ROM (study 4; Hoge et al., 2010). It was seen that using a production intensity method, the ROM and the torque performed for the SIS scores of 80, 60, and 40 were higher after the stretching protocol. Using the estimation method, the SIS score was also higher at angles of R80, R60 and R40 after stretching. This reinforces the validity of SIS in assessing stretching intensity.

The present SIS is expected to be useful in future studies of various different purposes, such as observing acute and chronic adaptations induced by different types of stimuli or to compare different types of populations. However, it must be considered that the SIS was only validated and tested for reliability in one human joint (i.e., knee), for a slow stretching maneuver, in a specific population (i.e., men with low knee extension flexibility). The SIS should be tested under other stretching conditions (e.g., different joints) and extended to other populations. In addition, the SIS should be also compared to other existing scales (e.g., Borg CR-100).

In the present study, a new and valid instrument with high reliability was developed to measure the perception of stretching intensity. Previous works have shown that stretching with different intensities induces distinctive physiological and performance effects. Accordingly, this scale is thought to be relevant to assessing stretch intensity. We expect that this instrument will be useful for clinical interventions or research settings.

Study 3 – Reliability of *in-vivo* sonographic biceps femoris (long head) muscle architecture assessment.

Design

A test-retest study was designed to assess BF muscle thickness, muscle length, fascicle length and fascicle angle reliability in the resting condition. 6-cm width images were captured as described in detail below, and muscle thickness, fascicle angle and fascicle length were measured. The image was subsequently cropped to a width of 3 cm and the fascicle length re-measured, enabling the reliability to be determined for a smaller imaging window. All measurements were captured across three sessions separated by at lest 20-min rest (Figure 18); one trained examiner (1) collected the images in sessions 1 and 2, and the other trained examiner (2) in session 3. Examiner 1 digitized the images in sessions 1 and 3, and examiner 2 digitized the images in all sessions. Ten men and ten women with no history of lower-limb injury or inflammatory conditions volunteered for the study (Table 2, page 41), with 10 of the participants (5 men and 5 women) completing the three sessions in order to allow determination of inter-examiner reliability.

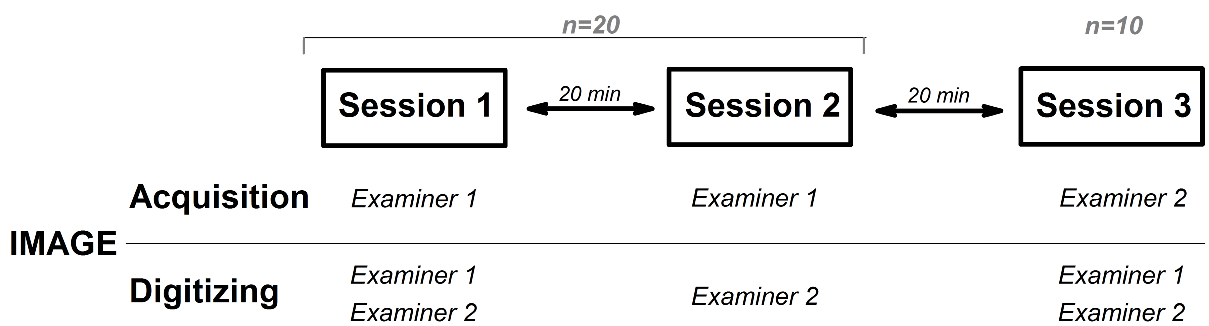

Figure 18. Study 3 design.

Procedures

BF considerations

The ultrasound assessment technique used in the present study was performed with consideration of BF muscle morphology and architecture (Figure 19). The biceps femoris long head is a bi-articular muscle that crosses both knee and hip joints and cadaveric studies have reported that BF has a non-uniform muscle architecture (Chleboun et al., 2001; Kellis et al., 2009; Kellis, Galanis, Natsis, & Kapetanos, 2010). In the resting (non-contracted) condition, fascicles are curved and oriented in three planes and therefore difficult to visualize in their entirety in a single sonographic image using a conventional linear ultrasound probe. Moreover, previous studies have reported that BF has a longitudinal mid-muscle aponeurosis that extends from the proximal muscle-tendon junction to the distal tendon-junction, which is visible in ultrasound images and onto which superficial fascicles insert (Kellis et al., 2009) (Figure 3). However, this aponeurosis presents a non-linear path in the resting condition even though the superficial BF aponeurosis follows a linear path for most of length of the muscle belly. Moreover, the proximal and distal BF muscle-tendon junctions (MTJ) are different in shape (Figure 19-B and C). The distal BF MTJ is superficial, close to the skin, and its most distal point is easily observed (Figure 19-B), whereas the distal BF MTJ ends more proximally compared to the biceps femoris short head. At the proximal site, the BF MTJ is located deep and merges medially to the semitendinousus tendon, that together insert onto the ischial tuberosity (Figure 19-C). Thus, the most proximal site of proximal MTJ is less visible than the distal MTJ.

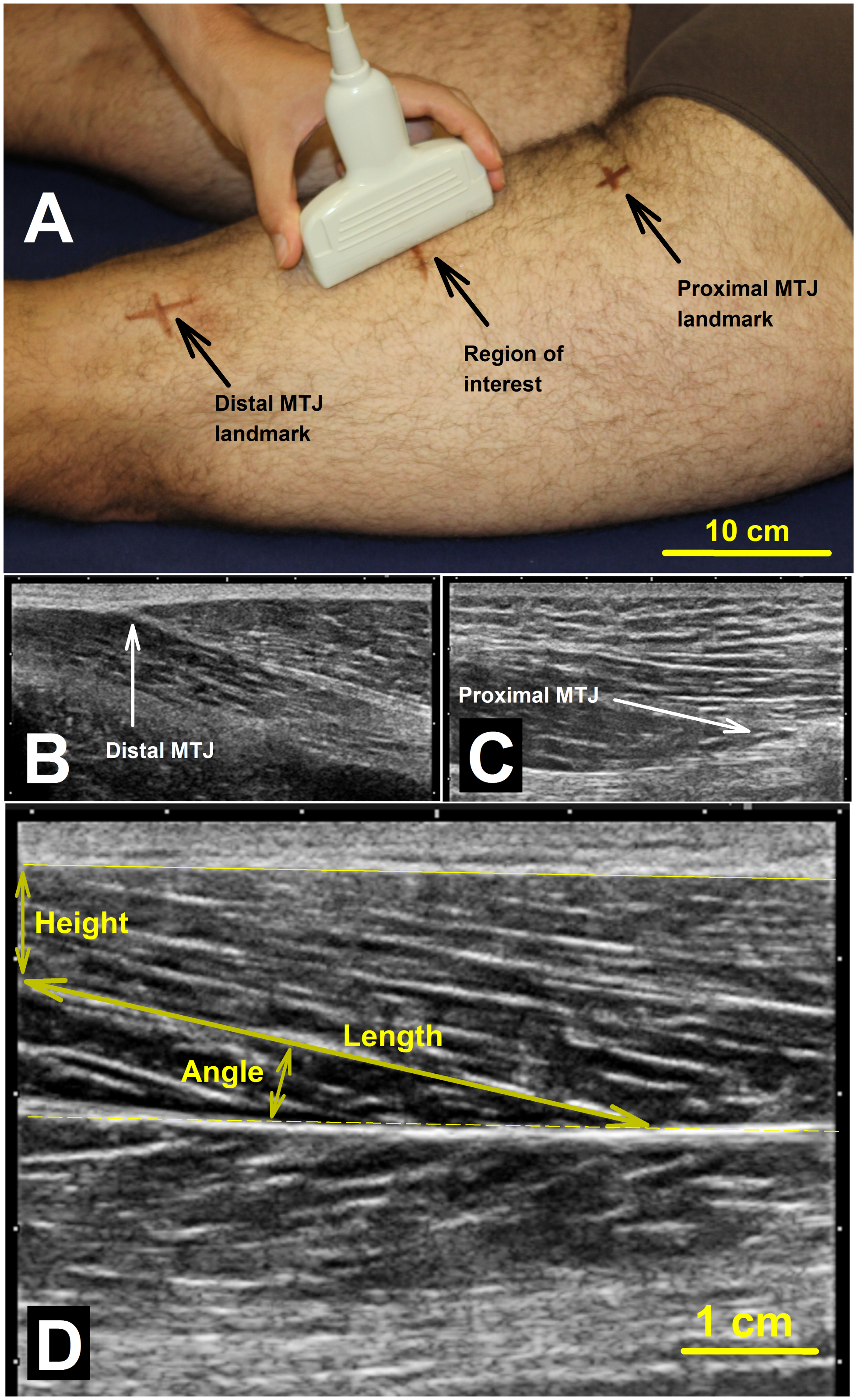

Figure 19. Procedures used to assess biceps femoris (long head) fascicle length and fascicle angle: determination of muscle-tendon junctions (proximal and distal) and region of interest to assess muscle architecture on the skin (A); distal (B) and proximal (C) muscle-tendon junctions; longitudinal ultrasonographic image of fascicle length and fascicle angle, on the region of interest (D).

Image acquisition

Before measurements were taken for the present study, each examiner performed more than 100 ultrasound trials. An ultrasound device (EUB-7500; Hitachi Medical Corporation, Chiyoda-ku, Tokyo, Japan) in B-mode with a 6-cm 10 MHz linear probe was used. The participants rested supine on a table after arrival at the laboratory. After 5 minutes of rest, initial scans were completed to identify BF morphology and specifically locate the proximal and distal MTJs. For distal MTJ identification, the probe was oriented perpendicular to the long axis of the muscle and the scanning probe was gradually moved distally. When the smallest muscle section was observed, the probe was oriented longitudinally at the intersection of superficial and deep aponeuroses (Figure 19-B) and a mark was drawn on the skin using a semi-permanent marker pen (Figure 19-A). The same procedure was used to identify the proximal MTJ, but the probe was gradually moved proximally before being reoriented longitudinally to the muscle when the MTJ was observed. Confirmation of skin marker placement was performed 3 times at each MTJ by moving the probe along the muscle to check whether the markers corresponded to the observable MTJ in the sonographic image. After confirmation, the distance between skin markers was measured with an anthropometric tape, as a BF muscle length outcome. These procedures were performed in all sessions.

The specific region for examination was chosen by scanning the mid-muscle region in both transverse and longitudinal planes until the image most clearly capturing the superficial and mid-muscle aponeuroses and BF fascicles was obtained. This position was slightly proximal to the position used in the study by Potier et al. (2009), and represented the region of muscle with the greatest muscle thickness and most visible fascicles. Because BF fascicles are longer than the imaging window, the ultrasound probe was oriented in line with the BF fascicles inserting onto the mid-muscle aponeurosis and perpendicular to the skin (Kwah et al., 2013). A mark was drawn on the skin to represent the site of the region for examination and the distance to the distal MTJ mark was recorded. In the following sessions, the relative distance between MTJ marks was used to re-identify this region. After measurement, the marks were erased and a minimum of 20 min was given between test sessions. In each session three images were taken that best verified the fascicle orientation and their insertion to the mid-muscle aponeurosis.

BF architecture digitizing

All sonographic images were processed using ImageJ software (NIH, 1.47v, USA). A trigonometric linear method described elsewhere (Noorkoiv et al., 2010) was used to determine fascicle length and fascicle angle. In all measurements, fascicle length (FL) was calculated using the equation: $FL = L + (h/\sin\beta)$, where L is the observable fascicle length from the mid-muscle aponeurosis to the most visible end-point,

h is the distance between the superficial aponeurosis and the fascicle visible distal end-point, and β is the angle between the fascicle (drawn linearly) and the superficial aponeurosis. In each image, three distinct fascicles were tracked and the mean value was calculated and used for statistical tests. In addition, muscle thickness was measured as the distance between the superficial and the mid-muscle aponeuroses at three points on the image (proximal, middle and distal). The mean of the three thickness measures were used to calculate the overall muscle thickness. The architecture measures were averaged for the three images taken in each session assessment, as a representative participants outcome. All images were numbered so the examiners could not identify the participants or image session.

Statistical analysis

Procedures similar to Noorkoiv et al. (2010) were used for statistical analyses. Intra- and inter-examiner image acquisition reliabilities were determined by comparing the muscle length measurements (i.e. distal to proximal MTJ distance). After confirming normality of data distribution, non-paired t-tests were used to compare the muscle length between men and women. Intra- and inter-examiner image digitizing reliabilities were determined for full- (6 cm) image window. The intra-examiner digitizing reliability was assessed using the coefficient of variation [CV% = (FL mean / standard deviation) × 100] after processing three single images 10 times. Inter-examiner digitizing reliability was assessed using intraclass correlation (ICC) and Pearson (r) correlations coefficients by comparing measurements between two examiners, each digitizing the same 10 6-cm wide images. Intra- and inter-examiner of the combined and separated image acquisition and digitizing process were determined for both full- and half-width (3 cm) image windows. The mean architecture values were calculated for the three images taken in each session assessment, and used for statistics. Intra-examiner reliability (n=20) was determined by comparing the values of two sequent assessments. Inter-examiner reliability (n=10) was determined by comparing BF variables measured by the two examiners. Intra-examiner reliability for both acquisition and digitizing (i.e. different image acquisition and data digitizing examiners) was determined by comparing results between sessions 1 and 3 (n=10). ICCs with 95% confidence interval (1-way random effects model) and the root mean square error (RMSE) were calculated for intra- and inter-examiner assessments using the 6-cm width window. The minimal detectable difference (MDD) was calculated at 95% confidence interval as $1.96 \times \sqrt{2} \times$ standard error of measurement (SEM) (Weir, 2005), using the intra-examiner data (n=20). The SEM was calculated as standard deviation of the difference scores divided by $\sqrt{2}$ (Weir, 2005). RMSEs and MDDs of all parameters assessed were normalized to the mean values. The estimated minimal sample for an effect size (ES) corresponding to the MDD values were determined based on Cohen´s paired t-test table for a 0.80 statistical power value (Cohen, 1988). ICCs were classified as:

"little" (0.00–0.25), "low" (0.26–0.49), "moderate" (0.50–0.69), "high" (0.70–0.89), and "very high" (0.90–1.00) (Domholdt, 2005). Values are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$.

Results

Image acquisition reliability. High intra- (ICC = 0.93 [0.82-0.97], r = 0.92) and inter-examiner (ICC = 0.90 [0.67-0.98], r = 0.90) reliabilities were found for muscle length measurements (i.e. proximal to distal MTJ). BF was significantly (P=0.02) longer in men (26.2 ± 2.3, n=10) than women (24.0 ± 1.5 cm, n=10). The probe was placed at 55.8 ± 6.6% of the distal-to-proximal muscle length, and no significant differences were observed between men (55.5 ± 6.0%) and women (56.0 ± 7.5%) (P = 0.88).

Image digitizing reliability. The CV% for digitizing the same sonographic images was very low (i.e. reliability was high) for fascicle length (1.1-1.6%), fascicle angle (1.1-1.7%), and muscle thickness (0.2-0.7%), respectively, for examiner 1. For examiner 2, CV% was also very low for fascicle length (1.0-1.6%), fascicle angle (1.2-1.8%), and muscle thickness (0.3-1.0%), respectively. The inter-examiner digitizing reliability was high for fascicle length (ICC = 0.79 [0.36-0.94]; r = 0.80), fascicle angle (ICC = 0.86 [0.55-0.96]; r = 0.86), and muscle thickness (ICC = 0.99 [0.98-0.99]; r = 0.99).

Examiner reliability. The mean (±SD) values and reliability of BF architectural measurements are presented in Table 9. For the full image width (6 cm), high to very high intra-examiner reliabilities were observed for most variables (ICC = 0.79-0.95, r = 0.79-0.95), and moderate to very high inter-examiner reliabilities were also observed (ICC = 0.56-0.92, r = 0.70-0.93). For the half image width (3 cm), high to very high intra-examiner reliabilities were observed for most variables (ICC = 0.79-0.93, r = 0.79-0.93), whilst moderate to very high inter-examiner reliabilities were observed (ICC=0.63-0.96, r= 0.89-0.98). The RMSEs for intra-examiner assessments were 9.4 ± 6.0 mm (9.8 ± 6.3%), 1.8 ± 1.1° (14.8 ± 8.7%), and 1.0 ± 1.0 mm (5.0 ± 6.6%) for fascicle length, fascicle angle, and muscle thickness, respectively. For inter-examiner assessment, RMSEs were 16.3 ± 12.1 mm (15.4 ± 11.5%), 2.9 ± 2.0° (25.6 ± 18.1%), and 1.4 ± 1.2 mm (5.1 ± 6.0%) for fascicle length, fascicle angle, and muscle thickness, respectively.

Table 9. Intra- (n=20) and inter-examiner (n=10) reliability outcomes for the biceps femoris (long head) architectural parameters.

	Full image (6 cm)							
	Intra-examiner (n=20)				**Inter-examiner (n=10)**			
	Session 1	**Session 2**	***r***	**ICC (CI)**	**Session 1**	**Session 3**	***r***	**ICC (CI)**
FL (mm)	96.4±17.9	96.5±16.9	0.79	0.79 (0.55-0.91)	99.9±15.6	111.9±23.4	0.70	0.56 (-0.01-0.87)
FA (°)	12.5±3.4	12.0±3.8	0.80	0.80 (0.56-0.91)	12.7±3.23	9.8±2.6	0.76	0.51 (-0.12-0.86)
DT (mm)	19.0±4.0	19.1±4.2	0.91	0.92 (0.80-0.97)	19.9±4.2	20.7±3.9	0.93	0.92 (0.72-0.98)
MT (mm)	20.3±3.7	20.4±3.8	0.95	0.95 (0.88-0.98)	21.0±4.0	21.4±3.7	0.87	0.88 (0.59-0.97)
PT (mm)	19.9±4.0	19.9±4.2	0.94	0.95 (0.87-0.98)	20.3±4.2	19.9±4.0	0.87	0.87 (0.58-0.97)
OT (mm)	19.8±3.8	19.8±3.9	0.94	0.94 (0.86-0.98)	20.4±4.0	20.7±3.8	0.90	0.91 (0.68-0.98)
	Half image (3 cm)							
	Session 1	**Session 2**	***r***	**ICC (CI)**	**Session 1**	**Session 3**	***r***	**ICC (CI)**
FL (mm)	94.7±16.6	97.1±19.2	0.79	0.79 (0.54-0.91)	102.9±16.45	120.3±25.0	0.90	0.63 (-0.10-0.91)
FA (°)	12.9±2.4	12.7±3.2	0.79	0.77 (0.50-0.90)	12.3±2.8	10.7±2.7	0.91	0.77 (-0.05-0.95)
DT (mm)	20.0±3.9	19.9±4.5	0.91	0.90 (0.77-0.96)	20.9±4.2	21.8±4.0	0.98	0.96 (0.54-0.99)
PT (mm)	20.1±4.1	20.1±4.5	0.90	0.90 (0.77-0.96)	20.8±4.5	21.0±3.8	0.89	0.89 (0.62-0.97)
OT (mm)	20.1±3.9	20.0±4.3	0.93	0.93 (0.82-0.97)	20.8±4.3	21.4±3.9	0.96	0.95 (0.82-0.99)

Legend: FL – Fascicle length; FA – Fascicle angle; DT – Distal thickness; MT – Middle thickness; PT – Proximal thickness; OT – Overall thickness.

Inter-examiner acquisition and digitizing reliability. Inter-examiner reliability results for both image acquisition and digitizing is are presented in Table 10. Fascicle length results were classified as moderate for full image (6 cm) and low for half image (3 cm) window widths. Similar results were obtained for fascicle angle. Muscle thickness results here classified as high to very high in in for both full and half image assessments.

Table 10. Inter-examiner image acquisition and digitizing reliability outcomes (n=10) for the biceps femoris (long head) architectural parameters.

	Inter-examiner and digitizing reliability (n=10)							
	Full image (6 cm)				**Half image (3 cm)**			
	Examiner 1	**Examiner 2**	***r***	**ICC (CI)**	**Examiner 1**	**Examiner 2**	***r***	**ICC (CI)**
FL (mm)	104.7±19.1	99.9±15.7	0.67	0.65 (0.12-0.90)	120.3±25.0	138.9±30.7	0.67	0.28 (-0.12-0.71)
FA (°)	11.6±2.6	12.3±3.2	0.71	0.70 (0.20-0.92)	10.7±2.7	9.9±3.0	0.63	0.47 (-0.11-0.83)
DT (mm)	21.9±5.9	19.9±4.2	0.87	0.78 (0.32-0.94)	21.8±4.0	21.6±3.8	0.93	0.91 (0.70-0.98)
MT (mm)	21.7±4.4	21.0±4.0	0.86	0.86 (0.55-0.96)	-	-	-	-
PT (mm)	19.9±4.3	20.3±4.2	0.83	0.84 (0.49-0.96)	21.0±3.8	20.8±3.9	0.84	0.84 (0.48-0.96)

Legend: FL – Fascicle length; FA – Fascicle angle; DT – Distal thickness; MT – Middle thickness; PT – Proximal thickness; OT – Overall thickness.

Minimal detectable difference, effect size, and sample size. The MDD was 8.4 mm (8.7%), 1.5° (11.4%), and 1.6 mm (6.6%) for fascicle length, fascicle angle, and muscle thickness, respectively. These MDD values corresponded to an ES of 1.01, 0.97, 1.44, for fascicle length, fascicle angle, and muscle thickness, respectively. Based on these ES values, it was observed a minimal sample size of 13, 13, and 7, for fascicle length, fascicle angle, and muscle thickness, respectively.

Discussion

Biceps femoris long head (BF) muscle architecture has been examined earlier, however a detailed report of the reliability of assessment has not been previously presented (Chleboun et al., 2001; Kellis et al., 2009, 2010; Potier et al., 2009). Chleboun et al. (2001) reported a high inter-examiner reliability (ICC=0.87), whilst Kellis et al. (2009; 2010) compared and characterized cadaveric versus ultrasound measurements in order to provide method validation. More recently, Lima et al. (2014) reported ICC values ranging from 0.78 to 0.99, without specifying the precise variables for which the statistics related, although fascicle length reliability had an ICC of 0.78 (it is not clear if this was for the whole procedure or the digitizing only). Also, it was not mentioned if measurements considered the existence of the mid-aponeurosis. In addition, Timmins et al. (2014) reported a very high reliability (ICC>0.97), but without detailing the digitizing procedures. In the present study the reliabilities of all architecture variables varied between intra-examiner (ICC = 0.79 and 0.95) and inter-examiner (ICC = 0.56 and 0.92) comparisons for the analyses using the full image window width. The RMSEs for different variables varied between 5.0 ± 5.2% and 14.8 ± 8.7% for intra-examiner assessments, and between 5.1 ± 6.0 and 25.6 ± 18.1% for inter-examiner assessments. Such values are in accordance with previous studies using sonography (Kwah et al., 2013), suggesting that errors from measurements do not depend on muscles studied but other factors (e.g. examiner assessment procedures). Fascicle length, fascicle angle, and muscle thickness measurement reliabilities were similar to those reported previously (Chleboun et al., 2001; Kellis et al., 2009, 2010; Lima et al., 2014; Potier et al., 2009; Timmins et al., 2014). In the present study, values of 9.6 ± 18.0 cm, 12.4 ± 2.8°, and 2.0 ± 0.4 cm, were obtained in full image window for FL, PA and MT, respectively, whilst in previous studies values of 5.9 ± 0.3 to 8.8 ± 1.8 cm, 14.9 ± 3.3 to 23.9 ± 3.8°, and 1.3 ± 0.2 to 2.7 ± 0.3 cm were reported. Thus, there appears considerable variability in results reported by different researchers, which can most probably be explained by the use of different sonographic, image digitization and variable calculation methods. In order to allow a better comparison between studies in the future, it would be ideal to develop a single method that could be used commonly by all researchers. This variability might also be related to the different participants lower limb length, and thus affecting the BF muscle size. However, because previous studies do not report the femur or thigh length it is not possible to compare the normalized values.

Among the different ultrasound approaches to assess muscle architecture, the panoramic extended-field-of-view method has been pointed as a recent, valid, and reliable tool to assess muscle fascicles that cannot be totally viewed from its insertions in the aponeurosis (Noorkoiv et al., 2010). However, this technique is not available to many researchers, and the complex architecture of BF makes difficult to use EFOV imaging; in particular, the rotation of fibers through the muscle, and the curved surface of the thigh, increases the difficulty in following the fascicular paths during the scan. Thus, we decided to use the single, still image acquisition technique (with extrapolation for the estimation of fascicle length) in the present study. Still, several difficulties and limitations should be considered with respect to the current methodology. First, the proximal biceps femoris muscle-tendon junction was harder to locate than the distal junction. This was due to the fact that muscle-tendon junction is deeper, and consequently less visible, and the proximal muscle-tendon junction has a non-uniform shape. According to Woodley & Mercer (2005) the proximal BF tendon inserts in the lateral side of the ischial tuberosity (one quarter of tuberosity surface), and the semitendinous tendon in the medial side (three quarters of tuberosity surface). In addition, the BF inserts laterally along the semitendinous until both tendons come together toward the ischial tuberosity. Consequently, given the complexity of proximal muscle-tendon junction shape, it becomes difficult to assess BF using ultrasound imaging. This finding is in agreement with previous studies (Kellis et al., 2009) where the investigators were unable to obtain clear and consistent ultrasound images. However, we have still found a high reliability for muscle length measurements for both intra-examiner (ICC = 0.93 [0.82-0.97], r = 0.92) and inter-examiner (ICC = 0.90 [0.67-0.98], r = 0.90) assessments, suggesting that the use of the methods presented in the current study should allow for reliable estimates of muscle length, and thus for the accurate replacement of the probe between testing sessions. In addition, the probe was placed at a 55.8±6.6% distance of the distal to proximal muscle length. The references for probe placement were based on the best site whereas the fascicles and aponeurosis could be better visible, and this was different from previously studies in which the references were bony landmarks. Indeed, since the that the BF has a non-uniform architecture (Kellis et al. 2010), the proposed BF architecture assessment method may not be appropriate if the aimed is to observe for changes in different locations of the muscle.

Another issue relates to the probe orientation with respect to the fascicular and aponeurotic paths. In theory, sonographic images should capture twin aponeurosis (i.e. the deep aponeurosis of the imaged muscle plus the aponeurosis of the muscle deep to it) as well as consistent and long white lines delineating the interspaces between fascicles (i.e. perimysia with adipose tissue and blood vessels) when assessing fascicle length. However, this is difficult to observe in the long head of biceps femoris because the fascicles are long, curved, and project in three planes in the resting condition (i.e. non-contracted muscle). Thus, a decision has to be taken to give a higher priority to probe orientation with respect to the fascicles or to their insertion onto the mid-muscle aponeurosis. Orientation according the aponeurosis may optimize the measurement of muscle thickness, however the fascicles will be less visible and this may decrease the accuracy and reliability of measurements. When the probe is orientated in accordance with the fascicles, their visualization is clearer but their insertion onto the aponeurosis, and thus the measurement of fascicle angle, may be negatively affected. In the present study, the criterion chosen was to clearly visualize the fascicle insertion onto the mid-muscle aponeurosis and thus allow a better fascicular imaging.

Another concern with the ultrasound method in the present study is related to the region of examination. We compared 6 cm to 3 cm images and did not find a notable difference for the intra- and inter-examiners reliabilities when the same examiners performed the image digitizing (see Table 9). However, when both image acquisition and digitizing was performed by different examiners, a clearly decrease in reliability was noted in the 3-cm images (see Table 10). In addition, the fascicle length measurements were clearly higher in the 3-cm images when the image acquisition was performed by different examiners (i.e. inter-examiner reliability, see Table 9) and when digitizing was performed by different examiners (see Table 10). This suggests that smaller probes (e.g. 3 cm) may be suitable for use in repeated measures study designs if image acquisition and digitizing are performed by the same examiner, but not if examiners are not the same.

As suggested by previous authors, ultrasound assessment can be used for a general biceps femoris long head architecture assessment (Kwah et al., 2013), however this method may not have sufficient sensitivity to detect small changes in muscle architecture in response to acute (i.e. within-day) and chronic (i.e. between day) interventions. It was obtained MDD values that suggest that studies examining adaptations to physical training must have a difference of at least 8.4 mm (8.7%, ES=1.01), 1.5° (11.4%, ES=0.97), and 1.6 mm (6.6%, ES=1.44) for fascicle length, fascicle angle, and muscle thickness, respectively. Lower value differences should be examined with assessment methods of with less methodological error. Based on these MDD values, future studies should test for at least 13, 13, and 7 participants for the FL, FA, and MT variables, respectively, in order to obtain a meaningful statistical difference.

In conclusion, single, still-image ultrasonography can be used for the reliable assessment of BF architecture, although the best reliability was achieved when the image acquisition and digitizing were performed by the same examiner and the 6 cm imaging window width was used. Nonetheless, such a method may not be useful for purposes where the measurement sensitivity needs to be very high.

Study 4 – Responses to static stretching are dependent on stretch intensity and duration

Design

A quasi-experimental design was used to observe the effects of three stretching protocols with different intensities and time under stretch. The subjects visited the laboratory on four separate occasions to perform three stretching protocols with different intensities and durations (Figure 20). Stretch intensity was considered as a percentage of maximum tolerated joint passive torque. The intensity of stretch and the stretch duration of the three protocols were inversely manipulated: fifty percent of maximum tolerable torque and 180 s in each repetition (P50), seventy-five percent and 135 s (P75), and a maximum tolerable torque intensity and 90 s (P100).

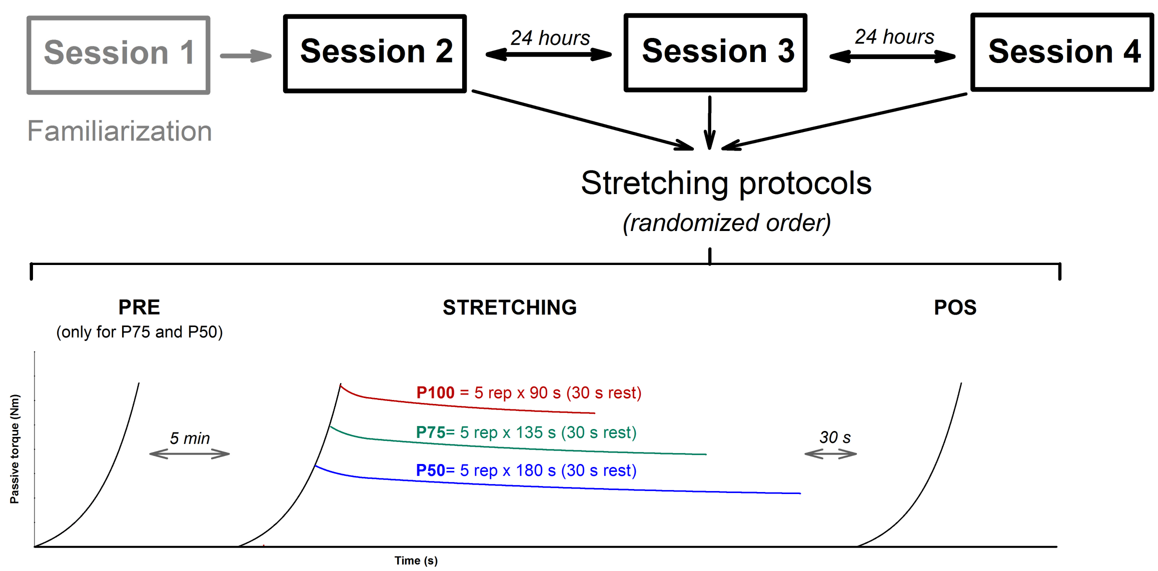

Figure 20. Study 4 design.

Protocol

In the first visit, the participants performed a familiarization session and anthropometrical assessment. In the remaining three visits, they performed a stretching protocol with different stretch intensities and time under stretch. Sessions were performed in a random balanced order and separated by at least 24 hrs. At the beginning of each experimental session, the skin was prepared for EMG, reflective markers were placed on skin, and the right ankle was immobilized in a static position with functional taping.

A passive knee extension testing setup for the right lower limb was used for this study (**Figure 6**, page 43).

The subjects did not perform any type of warm-up or stretching exercises before the experimental protocols. Briefly, subjects laid in a supine position, with the right hip flexed at 90° and left lower limb stabilized in a controlled and neutral position. The subject's right leg was firmly strapped by a Velcro to the arm of an apparatus that was fitted in a dynamometer shaft (Biodex System 3, Shirley, NY, USA) such that it could produce a passive knee extension. The right internal malleolus was aligned to the axis of rotation of the apparatus arm in all testing repetitions. All repetitions started with the apparatus parallel to the ground, in a manner that the leg could also be at 90° relative to the thigh.

Subjects were instructed not to move during the testing protocol, and to report for each stretching repetition the maximum knee range of motion tolerated without feeling pain or discomfort by saying "OK". An examiner stopped the apparatus upon the subject's signal and recorded the perception of stretching intensity at the beginning of the static phase on a VAS. The angular velocity of all the repetitions was set at 2°·s.

Intensity and time under stretch were manipulated in an inverse manner. Intensity was determined as a percentage of maximum tolerated stretch torque (i.e., peak torque) obtained in the first repetition. The intensity of P50 and P75 was determined by a preliminary repetition until maximum range of motion without pain or discomfort performed immediately before the stretching protocols. The corresponding angle to the percentage of the maximum torque was reproduced in further repetitions as a representative submaximal intensity. The following formula was used to determine the submaximal stretch intensity:

$$T_I = \left(P_T - T_{RP}\right) \times P + T_{RP}, \qquad (8)$$

where T_I is torque intensity (in Nm), P_T is peak torque (in Nm), T_{RP} (in Nm) is the torque measured at the initial testing positioning, and P is the percentage of stretching intensity. Five repetitions with a rest interval of 30 s between repetitions were performed in all protocols. The time under stretch for each repetition was 180, 135, and 90 s for P50, P75, and P100 protocols, respectively.

Maximal EMG activity of muscles tested were determined at the end of each session by subjects performing three maximal voluntary isometric knee extensor and knee flexor contractions (MVIC) for 5 sec with a 10-sec break.

Statistical analysis

All data were analyzed using IBM SPSS Statistics v20 (SPSS Inc., Chicago, IL). Absolute and relative (to first repetition of its protocol) values of peak torque, peak maximum angle, area under the curve (AUC) of dynamic phase, VAS score, and stress relaxation (SR) amplitude were determined at the end of 90-sec (i.e., peak torque less torque at 90-sec of static phase) and were used for comparisons between repetitions. To analyze the effects of each stretching condition on T-A curve, the torque at the percentiles of the T-A curve was compared between protocols. In order to compare the effects of stretch intensity on torque, comparisons were performed between repetitions for the same stretch duration. The repetition numbers compared between protocols were: 3rd of P50 vs. 4th of P75 (total stretch duration of 540 s), 2nd of P50 vs. 4th of P100 (360 s), and the 2nd of P75 vs. 3rd of P100 (270 s). Torque was compared at an angle corresponding to 40% of the peak torque of first repetition. Normal distribution was assessed with Shapiro-Wilk test. One-way repeated measures ANOVA followed by a post-hoc analysis with Bonferroni adjustment was performed for comparisons among repetitions, between protocols of the same repetition, and the same percentile of T-A curve between protocols. When normality was not confirmed, Friedman tests for repeated measures followed by Dunn test was used. For comparisons between only two repetitions, a t-test or Wilcoxon test was used, depending whether the variables had normal distribution or not. Statistical significance was set at $P<0.05$ for all tests.

Results

Experimental condition. No significant differences were found in ankle angle, hip angle, or thigh fixation between protocols ($P>0.05$). The T-A curve of the first repetition of each protocol was not significantly different among stretching interventions ($P>0.05$). The muscles EMG activities were bellow 3% in all trials.

Responses along stretching repetitions. The peak T-A responses during the repetitions of the stretching protocols are represented in Table 11. No significant differences in peak angle among repetitions were observed for P50 and P75 ($P>0.94$), but a significant difference was found for P100 in the fifth repetition compared to the second ($P<0.05$). No significant differences were observed in peak torque for P100 ($p>0.34$), but a significant decrease was found between the first and the remaining repetitions of P50 ($P<0.01$) and P75 protocols ($P<0.01$). The AUC did not significantly change among the P100 repetitions ($P>0.68$), but significantly decreased from the first to the remaining repetitions in both P50 ($P<0.01$) and P75 ($P<0.01$) protocols. The VAS score only increased significantly in the P100 protocol, between the first and the fifth repetition ($P=0.009$).

Table 11. Responses of maximum angle, peak torque, area under the curve, and VAS score during stretching repetitions of all protocols.

		Repetition				
	Protocol	**1**	**2**	**3**	**4**	**5**
Maximum angle (°)	**P100**	100	102.0±6.8 #5	104.9±9.2	105.8±10.1	107.9±10.3
	P75	87.1±3.8	87.4±2.9	88.8±3.4	87.3±3.4	87.2±3.2
	P50	71.7±4.2	71.7±3.9	72.2±4.1	71.8±4.2	71.7±4.8
Peak torque (Nm)	**P100**	100	99.8±16.6	100.5±18.8	103.5±20.8	107.4±23.1
	P75	71.1±6.0	66.9±5.8 #1,4,5	66.6±5.7 #1,5	64.0±5.5 #1	63.1±5.0 #1
	P50	47.4±3.6	43.6±3.8 #1	44.1±4.5 #1	42.2±3.9 #1	42.3±4.4 #1
AUC (Nm°)	**P100**	100	95.0±20.8	94.7±22.8	98.4±27.0	102.2±26.9
	P75	62.3±8.6	56.1±8.6 #1	54.8±8.6 #1	52.5±8.3 #1	51.6±7.5 #1
	P50	35.7±5.4	31.0±4.3 #1	31.1±5.0 #1,4	29.7±4.5 #1	29.9±5.4 #1
VAS score	**P100**	100	103.4±6.4	106.4±14.3	108.8±15.7	110.6±20.0 #1
	P75	83.0±22.0	81.8±29.8	84.1±35.4	84.8±46.0	85.1±42.0
	P50	67.6±54.8	59.7±41.9	62.2±52.1	63.5±48.7	67.8±38.3

AUC – Area under the curve. VAS – Visual analog scale.

Values (mean±SD) are normalized to the value of the first repetition. VAS values are expressed as median±IQR.

Significant difference from the repetition number (P<0.05).

The absolute and relative values of stress relaxation (SR) among repetitions are presented in Figure 21. Among protocols, absolute SR was found to be significantly different between P50 vs. P75 (P<0.01) and P50 vs. P100 (P<0.01) in all repetitions. No differences were found for P100 vs. P75 (0.07<P<1.00), except for the fifth repetition (P<0.05). Relative SR was not different between protocols in the first two repetitions (P>0.05). In the third repetition, a significant difference was found between P100 vs. P50 (P<0.01) and vs. P75 (P<0.01). In the fourth repetition, a significant difference was found between P50 vs. P75 (P<0.01) and P50 vs. P100 (P<0.01), and for the fifth repetition a difference was found for P50 vs. P100 (P<0.05). Among repetitions, a significant difference was found for relative SR between the first and the remaining repetitions in the P100 (P<0.05), P50 (P<0.01) and P75 (P<0.05), except for repetition 1 vs. 3 of both P50 (P=0.30) and P75 (P=0.31) protocols. In addition, repetitions 2 vs. 4 (P<0.01) and 2 vs. 5 (P<0.05) of P75 were also significantly different. In respect to absolute SR, the first repetition was significantly different to other repetitions in P50 (P<0.001), and P75 (P<0.05), except for repetitions 1 vs. 3 of P50 (P=0.29). In P100, only a difference between repetitions 1 vs. 3 was found (P<0.01). In addition, significant differences were found between repetitions 2 vs. 4 (P<0.01), 2 vs. 5 (P<0.01), and 3 vs. 5 (P<0.05), for the P75.

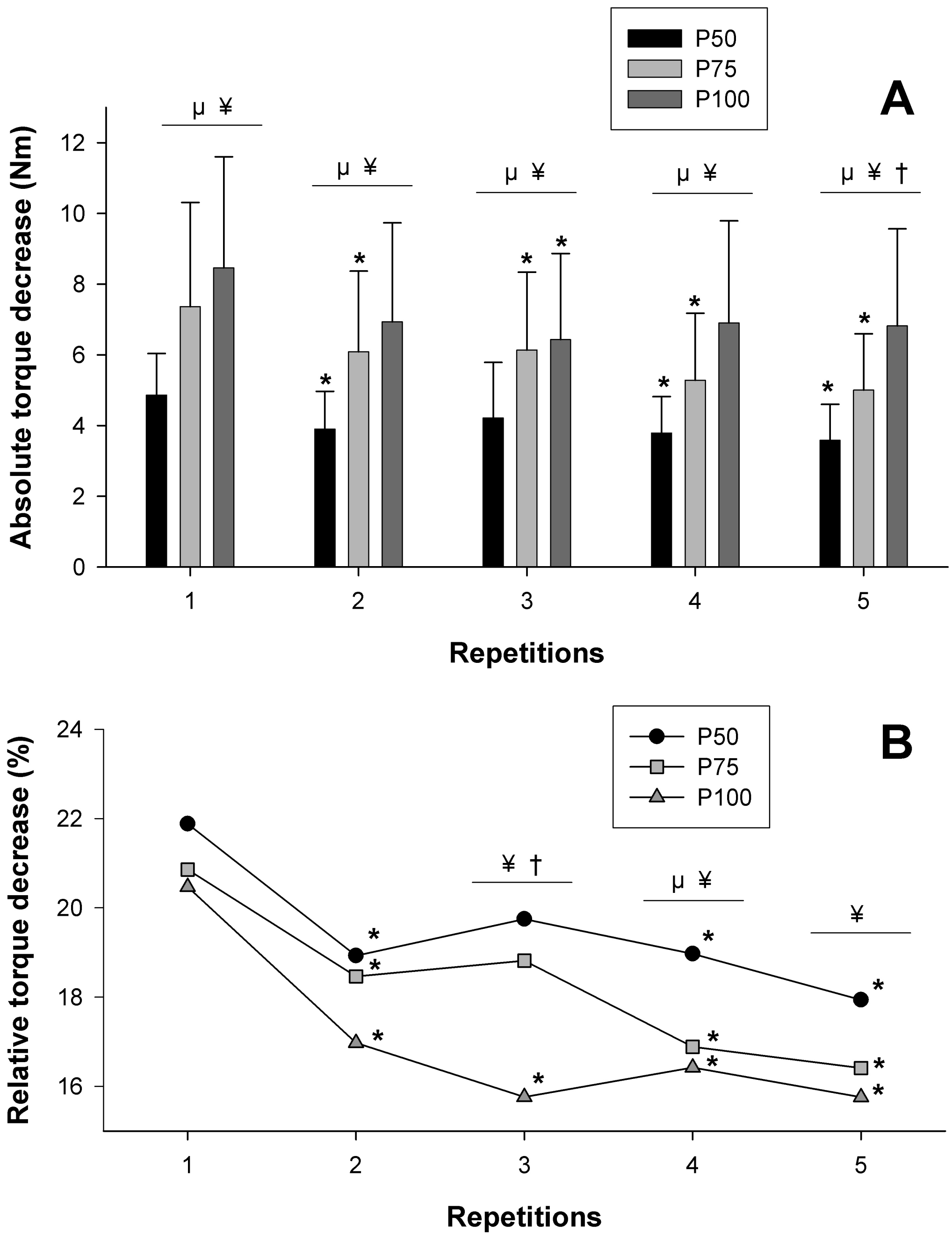

Figure 21. Absolute (A) and relative (B) stress relaxation (SR) in the repetitions of all protocols. Values are presented as mean±SD for absolute SR and mean for relative SR (values normalized to peak torque).

μ - Significant difference between P50 vs. P75 (P<0.05).

¥ - Significant difference between P50 vs P100 (P<0.05).

† - Significant difference between P100 vs P75 (P<0.05).

* - Significant difference from the first repetition (P<0.05).

Post-effects on peak torque-angle

The effects of stretching protocols on peak T-A outcomes are presented in Table 12. A significant increase in peak angle, peak torque, and VAS was observed only for the P100 protocol ($P<0.05$). A significant decrease of the area under the curve was observed in the P75 and P50 interventions ($P<0.05$).

Table 12. Changes induced by the stretching protocols on peak torque-angle curve outcomes.

	P100	P75	P50
Peak angle (º)	14.5±11.2 *	4.0±7.6	1.8±8.5
Peak torque (Nm)	19.8±27.6 *	−3.4±13.0	−5.6±15.9
AUC (Nmº)	19.1±34.5	−8.3±17.6 *	−14.6±18.8 *
VAS score	11.5±21.1 *	4.3±6.8	8.3±12.8

AUC – Area under the curve. VAS – Visual analog scale.

Values (mean±SD) are normalized to the value of the first repetition. VAS values are expressed as median±IQR.

* Significant difference at $P<0.05$ between pre- and post-stretching interventions.

Post-effects on torque-angle curve

Passive torque decreased after all stretching interventions (Figure 22). The P50 had the highest torque decrease, with significant differences compared to the P75 ($P<0.05$) in the first five percentiles of T-A curve. No significant differences were found in the last five percentiles among protocols ($P>0.05$).

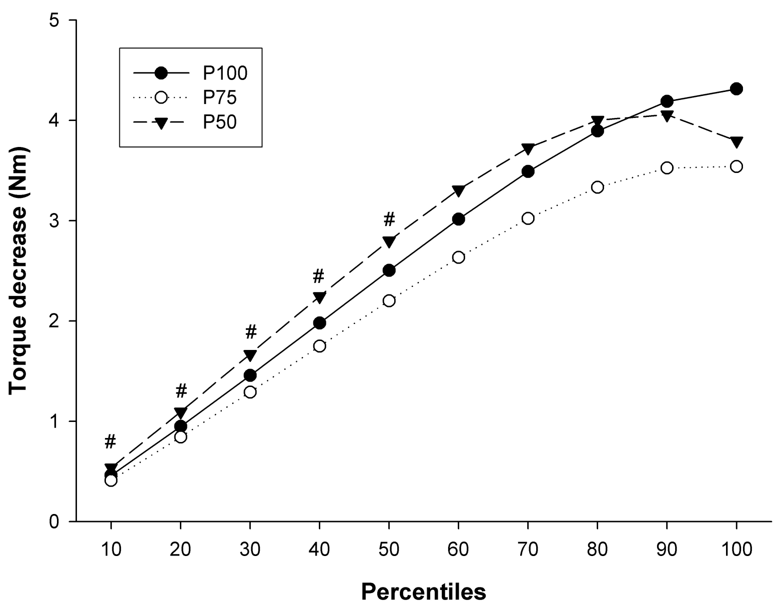

Figure 22. Effects of different stretching protocols on passive torque, in respect to the percentiles of maximum range of motion first repetition. Mean values are shown.

Significant difference at $P<0.05$ between P75 and P50.

When protocols were compared for the same time under stretch, no significant differences (P>0.05) were observed in torque change between stretching protocols (Table 13). Among repetitions of all protocols, it was observed that most of the torque decrease was obtained in the first repetition of P50 (mean of 87.6%) and P75 (89.0%) protocols, and in the second repetition of the P100 (85.8%) protocol.

Table 13. Comparisons between protocols for the torque change at 40% of peak torque of the first repetition, for a number of repetitions with the same time under stretch.

Torque at 40% of maximum torque	*540 s*	**P50**	**P75**	***p-value***
		3.4±1.4	3.4±2.0	0.99
	360 s	**P50**	**P100**	
		2.9±1.5	3.0±1.5	0.86
	270 s	**P75**	**P100**	
		3.3±1.9	2.8±1.4	0.27

The stretch duration of the protocols were: 540 s for P50(3)-P75(4), 360 s for P50(2)-P100(4), and 270 s for P75(2)-P100(3). Values in parentheses indicate the number of the repetition.

Stretching perception

A significant correlation between the relative values among repetitions was found (i.e., normalized to the value of the first repetition) of VAS score and peak angle (pearson=0.99, slope=2.14, y-intercept=−0.73, P<0.001), but not between VAS score and peak torque (pearson=0.85, slope=1.78, y-intercept=−1.14, P=0.06).

Discussion

Four main findings were found in the present study: 1) the perception of stretching intensity mainly changed according to knee angle alteration; 2) the protocols with sub-maximal stretching intensities did not increase peak torque-angle (T-A) outcomes, despite having more time under stretch; 3) the protocol with the highest time under stretch and lowest stretch intensity induced a higher passive torque decrease; 4) the change in T-A curve shape was different depending on the stretching intensity and duration.

In respect to the first finding, a significant increase was found in the knee angle only from the second to the fifth repetition of the P100 protocol. Previous authors have reported significant knee angle gains with similar number of repetitions, using a comparable stretching method (Boyce & Brosky, 2008). This joint angle increase was correlated with the change of the relative VAS score of stretching repetitions. The VAS score was not correlated to peak torque changes in the P100 condition, or the peak torque of sub-maximal protocols that decreased along repetitions. This result indicates the subject's perception of the stretching repetitions seems to be essentially determined by the degree of tissues extensibility (i.e., joint angle), and not by the tissue's tension (assuming that this tension is reflected on the joint passive torque measured). Such fact may be useful for clinical interventions. Still, because the VAS score has a high variability, a scale with specific properties to assess stretching intensity may measure the perception with more precision.

Second, different responses on T-A outcomes were found between protocols after stretching. Only the highest stretching intensity protocol induced gains on maximum angle, maximal tolerated stretch torque, and VAS score. The P50 and P75 did not change the peak T-A response. These results indicate that for the purposes of increasing joint range of motion, the intensity should be the highest as possible. The previous study of Walter et al. (1996) also supports these findings. In addition, the VAS score also changed in the P100, whereas it did not for the P50 and P75 conditions. This result also reinforces the previous suggestion that assessment of the perception of stretching intensity may be a useful tool for clinical interventions.

Third in respect to the passive torque change, the protocol with the lower stretch intensity and the higher stretch duration (i.e., P50) produced the highest torque decrease. To confirm whether this result was caused by either the stretch intensity or the stretch duration, comparisons between protocols were made for the same time under stretch (Table 13). No such differences among protocols were found. The torque decrement is related to the torque relaxation during stretch. In this study, the absolute stress relaxation at the end of 90 seconds was different between protocols. It was higher for the protocol with higher stretch intensity. This result is in agreement with previous literature (Fung, 1967). However, when this variable was normalized to peak torque the differences were much less evident. These differences between protocols were only found from the third repetition. Tian et al. (2010) concluded that the relative stress relaxation was not different between different lengths of gastrocnemius muscle and ankle angles. The results of the present study cannot advocate the same conclusion. In the present study we measured a global joint passive torque and not the passive tension of the tissues. It is necessary to investigate this hypothesis with more direct measures of tissues tension (e.g., supersonic shear wave elastography). Thus, because the highest torque decrease was seen for P50 protocol, it may be assumed that stretch duration seems to be the main factor for acute torque decrease. Previous studies also support this result (Light et al., 1984; McClure et al., 1994; Usuba et al., 2007).

Finally, the differences found in the torque of the T-A curve mainly happened in the beginning portion of the T-A curve. Such result is interesting because it indicates that the adaptation seems to be specific to the angle that the tissues are being stretched. In the previous study of Nordez et al. (Nordez et al., 2010), a similar conclusion was obtained for the mode of stretching (i.e., cyclic and static). In addition, previous studies have used the joint passive T-A curve to infer about structural adaptations after mechanical stimulus (Magnusson et al., 1996; McHugh et al., 1992; Nakamura et al., 2011; Ryan et al., 2008), by comparing the torque in a certain common angle. However, a criterion regarding which joint angle should be chosen for torque comparisons has never been reported. Considering the results of the present study, and the findings of Nordez et al. (2010), that passive torque response occurs differently along the T-A curve, we suggest data analysis should be performed for different angles of the T-A curve in order to assure the real adaptations to mechanical stimulus.

The clinical relevance of the present study targets the professionals that often use stretching interventions to increase knee flexors extensibility (e.g. hamstring muscle group). A tissue with higher stiffness has less capacity to store energy during the stretch (Magnusson et al., 2000), and consequently greater risk of rupture. Thus, increasing the compliance of the tissue will decrease the risk of rupture. The results of the present study suggest that stretching with higher intensity may increase energy store capacity during the stretch, and consequently that be beneficial to decrease the risk of injury (e.g. during eccentric contractions).

As a conclusion, the present study tested the effects of stretching protocols, in which the stretch intensity and duration variables were inversely manipulated. It was observed that a higher intensity stretch potentiates the acute joint range of motion gains, and a sub-maximal intensity and higher time under stretch potentiate passive torque decrement. In addition, different effects were observed in the passive torque–angle curve after the three protocols. Researchers and clinicians should consider this when applying different stretching interventions.

Study 5 – Are rest intervals between stretching repetitions efficient to acutely increase range of motion?

Design

An experimental study was conducted to determine the influence of resting between stretching repetitions on the joint torque-angle response (Figure 23). Participants came to the laboratory for three sessions to compare the performance between a stretching with rest intervals (RI) to a non-rest interval (NRI) protocol (see Figure 13, page 69).

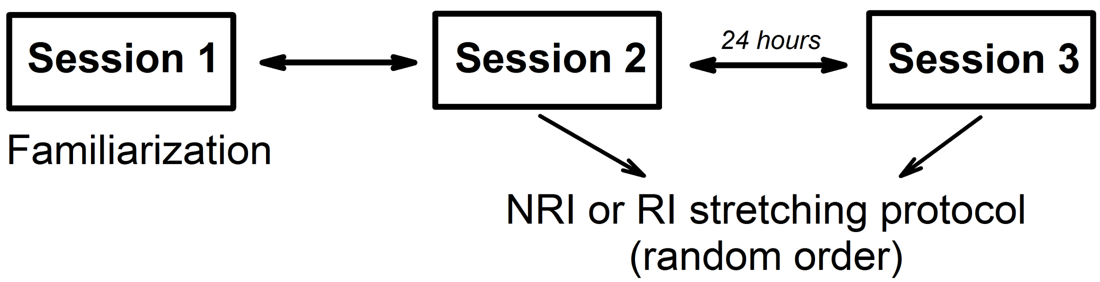

Figure 23. Study 5 design.

Protocol

In the participants first visit, it was performed a familiarization session. In the second and third visits, the participants performed the stretching protocols. Before each protocol, anthropometric measurements were obtained, procedures for EMG were done, reflective markers were placed, and the right ankle was fixed in a static position with a functional bandage. A three-hour interval separated these two sessions to dissipate stretch effects (Magnusson et al., 1996; Ryan et al., 2008). Participants did not perform any type of stretching exercises before or between the experimental protocols.

The participants performed a passive knee extension test on the right lower limb (**Figure 6**). Briefly, participants were in a supine position with their right thigh flexed to 90°, and the left lower limb fixed in neutral position. The leg was strapped to the arm of an apparatus that was fitted in a dynamometer shaft (Biodex System 3 research, Shirley, NY, USA), allowing passive knee extension movement. The right internal malleolus was aligned to a specific marker of the apparatus arm. All repetitions started with the leg positioned at 90° relative to the thigh. The angular velocity was set to 2°/s to mobilize the knee.

The two stretching protocols performed included: RI) a rest interval of 30-s between repetitions; NRI) no rest interval between repetitions. Protocols were conducted with a balanced order. Five repetitions were performed in the RI protocol, and the maximal tolerated number of repetitions was performed in the NRI protocol (Figure 24). The intensity for repetitions of both protocols were the maximal tolerable torque without pain. Due to the participants' different degrees of stretch tolerance, the maximal number of NRI repetitions varied. A previous pilot study (unpublished observations) showed that participants did not exceed a maximum of five NRI repetitions without pain. Participants were asked to report the maximal ROM without feeling pain by saying "OK" in all repetitions of both protocols. An examiner stopped the apparatus in response to the subject's signal. In both protocols repetitions of the static stretching were held for 90 s. In the NRI protocol, at the end of the 90 s of each NRI repetition, participants were asked whether they could increase the ROM without feeling pain. When participants said "yes," the ROM was increased to a new maximum until the participants said "OK." The maximal number of NRI repetitions was determined when the participants reported that they could not perform an additional repetition without pain. Thirty seconds after the end of each protocol, a repetition with no static stretching was performed to the maximal ROM. The protocols were compared for the same number of stretching repetitions to enable comparisons for the same stretch duration. Thus, four groups were analyzed: 2NRI (n=20), 3NRI (n=19), 4NRI (n=6), and all participants (n=47).

At the end of each session, the participants performed three maximal voluntary isometric muscle contraction (MVIC) repetitions for 5-s duration with a 10-s break for both knee extension and flexion, with the knee at 90°, for the purposes of EMG signal normalization.

Statistical analysis

The data were processed using IBM SPSS Statistics 19.0 (IBM Corporation, NY, USA) software. Data was normalized to the first repetition value of its protocol (i.e. baseline), and are reported as the mean and standard deviation (mean±SD). Normality was first checked using the Shapiro-Wilk test. The maximal ROM, peak torque (i.e. maximal passive torque), and the passive torque at a given angle were used for the statistical analysis. The passive torque was analyzed at a given angle common to all participants in the first repetition of stretching protocols and the repetition after the stretching. The knee angles corresponded to the angle percentiles of the less-flexible participant performance. Statistical analysis was completed for the same numbers of repetitions between both protocols, so stretch duration could be the equal. Thus, three sub-groups were considered for those who performed: two NRI repetitions (2NRI, n=20), three NRI repetitions (3NRI, n=19), and four NRI repetitions (4NRI, n=6). The initial ankle and hip angles at the beginning of the stretching protocols were compared using paired t-tests. A one-way repeated measures ANOVA [repetition (1, 2, 3, 4, 5, post)] was performed for all participants in the RI condition. For comparisons between repetitions of the 2NRI, 3NRI, and 4NRI sub-groups, two-way repeated measures ANOVA [protocol (NRI, RI) × repetition (1, 2, … , post)] followed by a post-hoc Bonferroni analysis was performed. The comparisons between 2NRI and for 4NRI sub-groups were performed using unpaired t-tests. Paired t-tests were used to compare between the protocols in the same repetition number. Comparison between the time conditions (pre- and post-stretching) for all participants (n=47) was performed using paired t-tests. Statistical significance was set at a p-value <0.05.

Results

No significant differences were found between the protocols for the ankle (*P*=0.61) and hip (*P*=0.82) angles. No differences were observed between protocols in the first repetition for submaximal torque in all percentiles (*P*>0.05), maximal ROM (2NRI: p=0.25; 3NRI: *P*=0.13; 4NRI: *P*=0.40), and peak torque (2NRI: p=0.86; 3NRI: p=0.26; 4NRI: p=0.51). The maximal number of repetitions (R) performed by the participants in the NRI protocol ranged between 2 and 5 (2R, n=20; 3R, n=19; 4R, n=6; 5R, n=2). A typical torque-angle and EMG response for both NRI and RI stretching protocols is depicted in Figure 24.

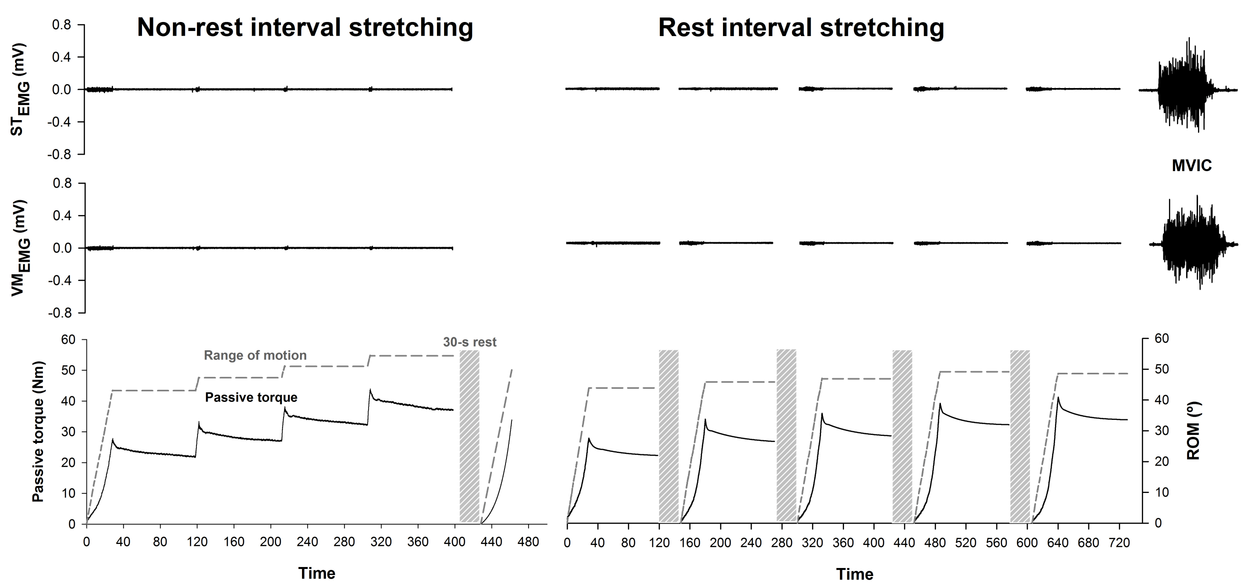

Figure 24. Typical range of motion, passive torque, and EMG muscles activity during stretching and during a MVIC (right top corner) from one participant (#29) during a rest interval (left) and a non-rest interval (right) stretching protocols.

The participant tolerated 4 NRI repetitions. In this case, the post effects between protocols were performed between the post NRI repetition, and the stretching phase of the RI fifth repetition.

Legend: ST – Semitendinosus; VM – Vastus medialis; MVIC – Maximal voluntary isometric contraction.

A significant effect for repetition was observed in the maximal ROM (p<0.0001) and submaximal torque (P<0.0001) but not for peak torque (P=0.12) during the RI protocol (Figure 25).

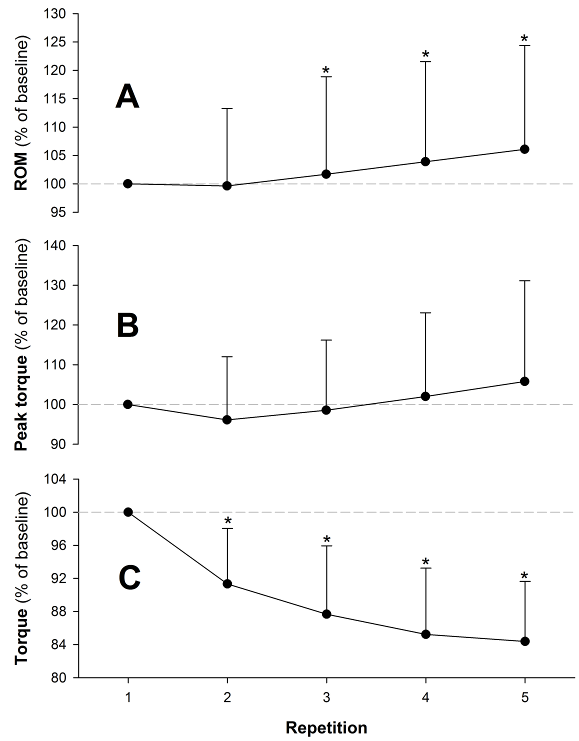

Figure 25. Range of motion (A), peak torque (B), and submaximal torque at the 90th percentile angle (C) responses during a rest interval (RI) stretching protocol with five repetitions.

Values are normalized to baseline condition (i.e. first repetition).

* - Statistical difference from baseline at p<0.05.

The maximal ROM and peak torque response during and after the NRI and RI stretching protocols are depicted in Figure 26. A significant protocol × repetition interaction for the maximal ROM and peak torque was observed in 2NRI (ROM: P=0.001; peak torque: P=0.018) and 3NRI (ROM: P=0.001; peak torque: P=0.00001) groups. A significant effect for protocol was observed for ROM in 2NRI (P=0.02), and 3NRI (P=0.0001) groups but not for the 4NRI group (P=0.07). A significant effect for protocol was observed for peak torque in 2NRI (P=0.04) and 3NRI (P=0.0001) but not for the 4NRI group (P=0.17). A significant effect for repetitions was observed for ROM in 2NRI (P=0.0001), 3NRI (P<0.00001), and for 4NRI (P=0.003) groups. A significant effect for repetitions was observed for peak torque in 2NRI (P=0.04), and 3NRI (P=0.003) groups, but not for 4NRI groups (P=0.09). For all participants, an increase of maximal ROM was observed in both protocols (NRI: +13.6±10.2%, P<0.000001; RI: +5.9±10.4%, P=0.0005) after stretching, however, the maximal ROM increase was significantly higher for NRI compared to RI (P=0.0001). The 4NRI group had a higher increase compared to the 2NRI on maximal

ROM (2NRI=7.2±7.1% vs. 4NRI=22.3±8.6%, p=0.0002) and peak torque (2NRI=1.4±15.3% vs. 4NRI=39.1±27.6%, p=0.0002).

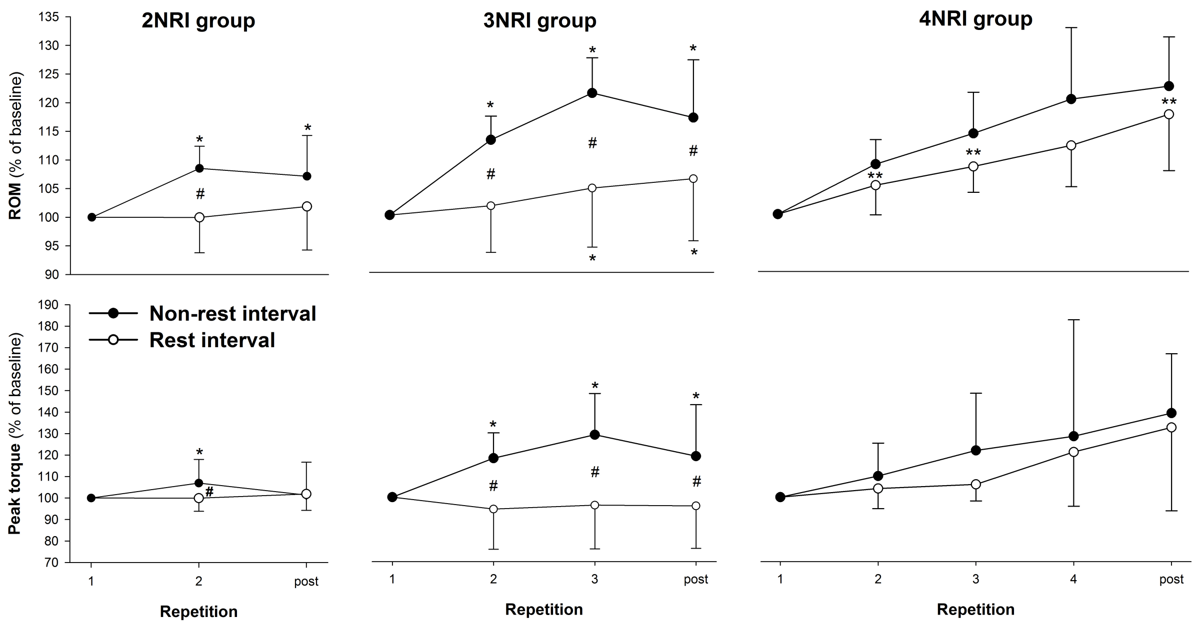

Figure 26. Range of motion (ROM) and peak torque responses in the 2NRI, 3NRI, 4NRI sub-groups during the stretching with and without rest interval between repetitions.

* - Statistical difference from baseline (p<0.05).

** - Statistical effect for repetition number compared to baseline (p<0.05).

- Statistical difference between protocols for the same repetition number (p < 0.05).

The percent change in submaximal passive torque of both protocols is depicted in Figure 27. A torque decrease in all percentiles was observed for both stretching protocols (P<0.05); however, the extent of the torque decrease in the initial component of the torque-angle curve tended to be lower in the NRI protocol (p-values ranged from 0.05 to 0.08 in the 10th to 60th percentiles).

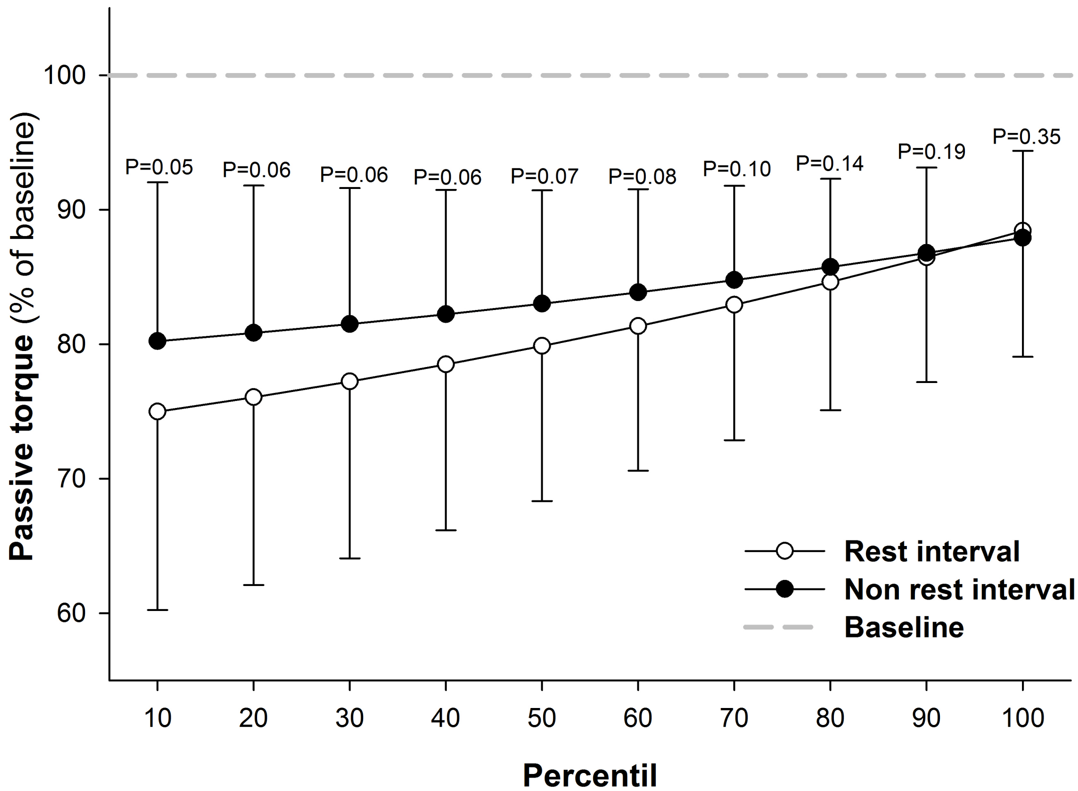

Figure 27. Effects of RI and NRI stretching protocols on submaximal torque for all participants.
Note: Torque is showed as a percentage of baseline condition, and the x-axis corresponds to the percentiles of maximal range of motion. All torque percentiles of both protocols are statistical different ($p<0.05$) from baseline. The p-values of paired t-test between the % torque decrease of RI and NRI stretching protocols are presented for each percentile.

The average EMG of ST and VM was less than 3% in both protocols. No significant differences were found between protocols for average EMG between protocols in both ST (P=0.54) and VM (P=0.96) muscles.

Discussion

The present study investigated the effects of non-resting (NRI) and resting (RI) between stretching repetitions on maximal ROM, peak torque, and passive torque at a given angle, as well as the minimal number of stretching repetitions required to change the maximal ROM, peak torque, and passive torque. The initial testing conditions were equal between the two protocols, indicating that participants were tested equally in both protocols. It was found that: 1) NRI stretching is advantageous for increasing the maximal ROM and peak torque, although the RI is better for submaximal torque decrease at a given angle; 2) the minimal number of static stretching repetitions varies depending on the stretching purpose (i.e., changing the maximal ROM, peak torque, or submaximal torque) in both RI and NRI protocols. The EMG activity was less than 3% of the MVIC during the stretching protocols. This value was lower than those previously reported when stretching to maximal ROM (Blazevich et al., 2013; Magnusson et al., 1996; Magnusson & Simonsen, 1996). Thus, it is assumed that muscle activity did not affect the comparisons between protocols.

Studies examining acute increases in joint flexibility often ask participants to perform maximal ROM without pain (Herda et al., 2011, 2012; McHugh & Cosgrave, 2010a). In the present study, participants were asked to perform the maximal ROM with no pain in all repetitions of both protocols; however, the NRI showed a higher ROM and peak torque increase during and after the stretching protocol than the RI condition. This greater increase was clearly noted at the second repetitions in all participants (i.e., NRI= +8.6±13.7% vs. RI= +1.3±6.9%). These results suggest that a rest interval should not be used if the objective is to acutely increase maximal ROM and torque tolerance. Previous studies have suggested that a higher stretching intensity is more effective for joint ROM gains (Magnusson & Simonsen, 1996; Walter et al., 1996). In the present study, the joint ROM increase during the stretching was higher for NRI than RI; consequently, a greater ROM was observed after the stretch (13.6±10.2% vs. 5.9±10.4%, respectively). The NRI was also shown to be more efficient than RI, by producing better results in a shorter time. For instance, in all participants, the NRI induced a ROM increase of 8.6±13.7% from the first to second repetition (i.e., 90 s of stretching time) compared to a 8.3±9.6% gain from the first to fifth repetition of the RI condition (i.e., 360 s of stretching time).

In addition, two repetitions for NRI (i.e., 180 s of stretch) and 3 for RI (i.e., 270 s of stretch) were seen to be necessary in order to significantly increase the maximal ROM after stretching, despite the increase of NRI being greater than RI. Regarding the effects on peak torque, changes were only observed after the NRI stretching with 3 repetitions. No changes were observed for 4 NRI repetitions, probably due to the small sample size (n=6). We consider this to be a study limitation. This means that the lack of rest between repetitions allows an increase of peak torque during stretching. Consequently, a higher peak torque increase can be observed after stretching, but not when resting between repetitions.

Moreover, the static stretching with constant torque (CT) and the PNF technique have been reported to be more effective than a constant angle static stretching with rest intervals to increase the maximal ROM (Herda et al., 2011; Magnusson & Simonsen, 1996; Yeh, Tsai, & Chen, 2005). However, it remains unknown whether the NRI method is more effective than the constant-torque method or the PNF for producing a ROM gain. With respect to the PNF method, considering the positive effect of muscle contraction on joint angle gains, the PNF contract-relax method is peculiar because it does not include rest between repetitions. In the present study, we observed a higher ROM increase for the NRI than for the RI during and after stretching. Thus, it is unclear which of the variables – i.e., `muscle contraction´ or `rest interval´ between repetitions – contributes more to the effectiveness of PNF. Further studies should be designed to compare the PNF and NRI methods in order to evaluate the contributions of muscle contraction in a stretching position.

In addition, the literature indicates that stretching at a higher intensity induces greater stress relaxation (i.e., torque decrease during static stretch) and lower passive torque (Herda et al., 2011; Magnusson & Simonsen, 1996). The results of the present study are somewhat contradictory to this finding. The highest intensity produced by the NRI method did not produce a greater decrease in torque than the RI protocol. Instead, there was a smaller decrement with no differences in EMG between protocols. The reason for this result is unknown. A speculative explanation of the lower torque decrement observed in the more intense stretching protocol might be related to calcium release mechanisms triggered by muscle damage, which may partially counteract the passive torque decrease (Whitehead, Morgan, Gregory, & Proske, 2003). Future studies should examine this possibility; however, one RI repetition for 90-s at maximal ROM was found to be sufficient to change the submaximal torque. Previous studies reached different conclusions; however, they used different stretching durations (Matsuo et al., 2013; Ryan et al., 2009). It is possible that the number of stretching repetitions required to change submaximal torque would depend on stretch duration (Boyce & Brosky, 2008).

To our knowledge, no previous studies have characterized the response to an NRI stretching protocol. It was observed that participants performed a different number of NRI repetitions. Thus, the comparison analysis between protocols was performed for different sub-groups with equal number of repetitions, so that stretch duration could be the same. The inter-participant NRI repetition variance might be related to the participant's stretch tolerance, which may be influenced by the sensitivity of the mechanoreceptors in the tissues being stretched and/or the meaning of the afferent information in the central nervous system.

A limitation of this study should be considered. The effect of the rest interval could not be fully tested, because this variable was not isolated. It was observed a greater joint load during the stretching in the NRI compared to the RI, for the same stretching duration. Thus, a future study should be performed to investigate the effect of rest interval with equal intensities among repetition; however, in practical terms, this study provides valuable information that could easily be used by coaches and athletes, unlike other proposals that also show advantages, though it is more difficult to execute (e.g., constant torque method) (Herda et al., 2011, 2012; Yeh et al., 2005).

Study 6 – Acute stretching effects on the joint passive torque-angle: high-intensity and short-duration vs. low-intensity and long-duration.

Design

An experimental study was conducted to compare the tome course effects between two stretching protocols with different intensities and durations on the joint torque-angle response (Figure 28). Participants came to the laboratory for three sessions to compare the performance between a stretching with high-intensity with short-duration (HISD) vs. low-intensity with long-duration (LILD) protocols. A passive knee extension test for the rigth lower limb was used (**Figure 6**).

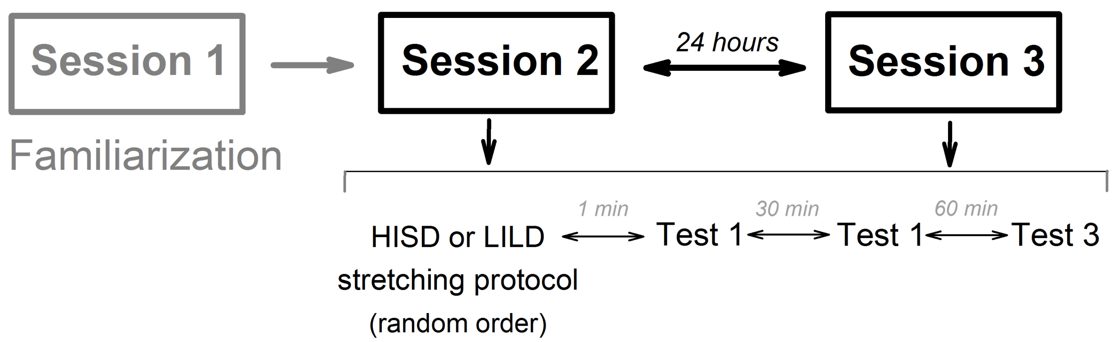

Figure 28. Study 6 design.

Protocol

On the first visit, a familiarization session was performed. In next two sessions, the two stretching protocols were performed with a balanced order and an interval time of at least 24 h between protocols. In the beginning of each session, the skin was prepared for EMG, reflective markers were placed, and the right ankle was immobilized in a static position with elastic tape. No warm-up or stretching exercises were performed before the stretching protocols. For the knee extension protocol (**Figure 6**), participants lay in a supine position with the right hip flexed at 90° and left lower limb stabilized in a neutral position. All repetitions started with the leg at 90° to the thigh. The angular velocity of all repetitions was set with 2°/s.

Stretch intensity was considered as a percentage of the maximum tolerated joint passive torque (PT). Two combinations of stretch intensity and stretch duration were studied (**Figure 29**): 1) 50% of PT and a duration of 900 s (LILD: low intensity and long duration), 2) 100% of PT with a maximum number of 90-s repetitions without rest intervals between repetitions (HISD: high intensity and short duration). A maximum number of repetitions without rest intervals between repetitions were performed on the HISD protocol in order to ensure that the maximum tolerable torque was achieved during the stretching. Thus, every 90 s of stretching, participants were asked if they could stretch further with no pain. If they agreed, the knee angle was increased to a new ROM. If not, the stretching was stopped (see example in **Figure 29**). Thus, the number of 90-s repetitions without rest interval varied across participants due to the different degrees of stretching tolerance. This protocol was chosen because we previously observed in a pilot study (unpublished data) that a non-rest interval protocol induces greater ROM and peak torque increases during the stretching than a conventional rest interval protocol. For the LILD protocol, a preliminary repetition performed to the maximal ROM was performed 5 minutes before the stretching protocol to determine the knee angle that corresponded to 50% of the peak torque. After both stretching protocols, a repetition to the maximal ROM without pain was performed at 1, 30, and 60 min after stretching to observe the time course effects. At the end of each protocol session, three repetitions of 5-s maximal voluntary isometric muscle contractions (MVIC) were performed for both knee extension and flexion, for the purposes of EMG signal normalization.

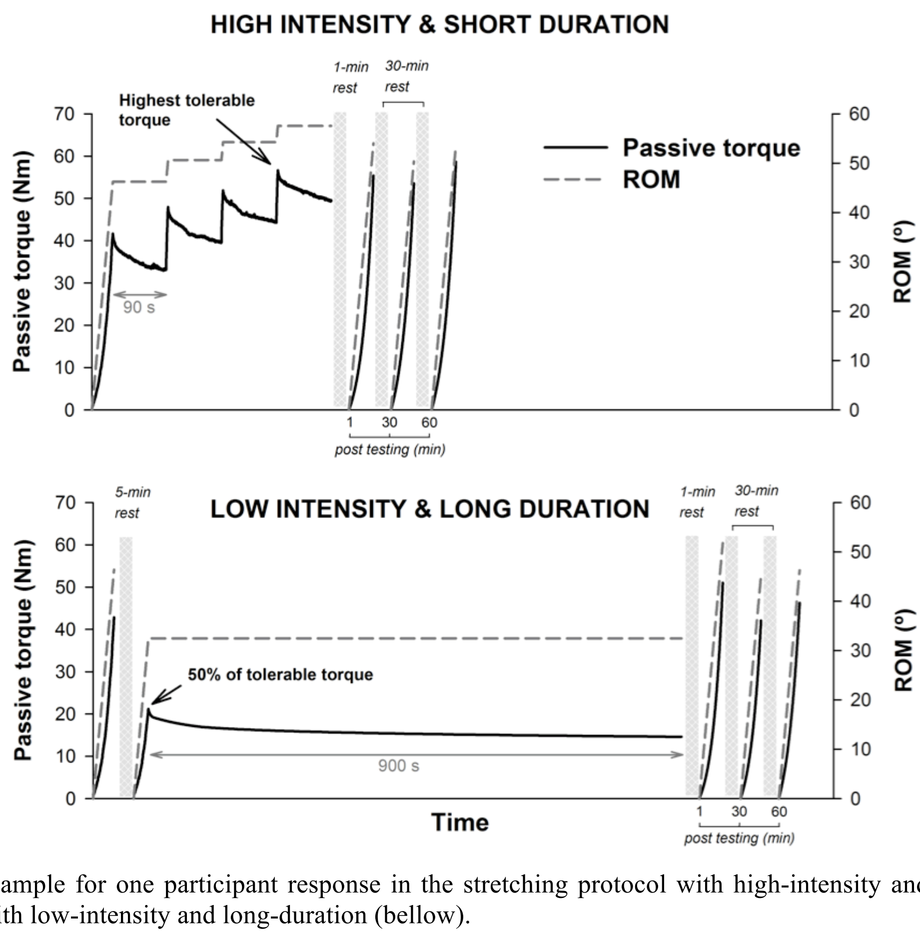

Figure 29. Example for one participant response in the stretching protocol with high-intensity and short-duration (above) and with low-intensity and long-duration (bellow).

Statistical analysis

Data were analyzed using IBM SPSS Statistics v20 (SPSS Inc., Chicago, IL). Normal distribution was assessed with Shapiro-Wilk test. All variables were first normalized to the baseline value (i.e., first repetition). The peak torque, maximal ROM and passive torque variables were used for analysis. The average ROM and peak torque of all HISD repetitions was determined to calculate the average intensity performed during the stretching. The passive torque was compared at ten given knee angles, which were determined based on the percentiles of maximal ROM performed by each participant in the first repetition (i.e., baseline). A two-way ANOVA [protocols (HISD, LILD) × torque percentile (10, 20, 30, 40, 50, 60, 80, 90, 100)] was performed for the absolute passive torque in first repetition of both protocols in order to confirm that participants were tested in the same initial condition. A two-way ANOVA [protocols (HISD, LILD) × time (pre, 1-min post, 30-min post, 60-min post)] wase performed for maximal ROM, peak torque and EMG. A two-way ANOVA [time (pre, 1-min post, 30-min post, 60-min post) × torque percentile (10, 20, 30, 40, 50, 60, 80, 90, 100)] was performed for the analysis of passive torque in each stretching protocol. These ANOVAs were followed by a post-hoc analysis with Bonferroni test when appropriate. Paired t-tests were performed to compare the passive torque in each percentile of ROM between protocols in the same testing time. Statistical significance was set at 0.05 for all tests.

Results

The number of repetitions in the HISD protocol varied between subjects (n=8 for 2NRI, n= 6 for 3NRI, n=3 for 4NRI). Thus, the stretch duration for HISD was 243.5±69.5-s. The average intensity during the HISD protocol was 109.2±10.4% of initial peak torque and 107.3±7.6% of initial maximal ROM. Neither a significant effect of protocols ($p=0.12$) nor a protocols × torque percentile interaction ($p=0.486$) was found for the torque percentiles on the first repetition; however, a significant effect was found for the torque percentile ($p<0.00001$). A typical example of the torque-ROM curves before and at 1, 30 and 60-min after the two stretching interventions is depicted in **Figure 30**-A.

The passive torque at the baseline percentiles before and at 1, 30 and 60 min after the two stretching protocols is depicted in **Figure 30**-B. A significant interaction (time × percentile) was observed for passive torque in both HISD ($p<0.00001$) and LILD (=0.003) protocols. A significant time effect was observed for passive torque in both HISD ($p=0.00001$) and LILD ($p=0.028$) stretching protocols. A significant percentile effect was observed for passive torque in both HISD ($p=0.01$) and LILD ($p<0.0001$) stretching protocols.

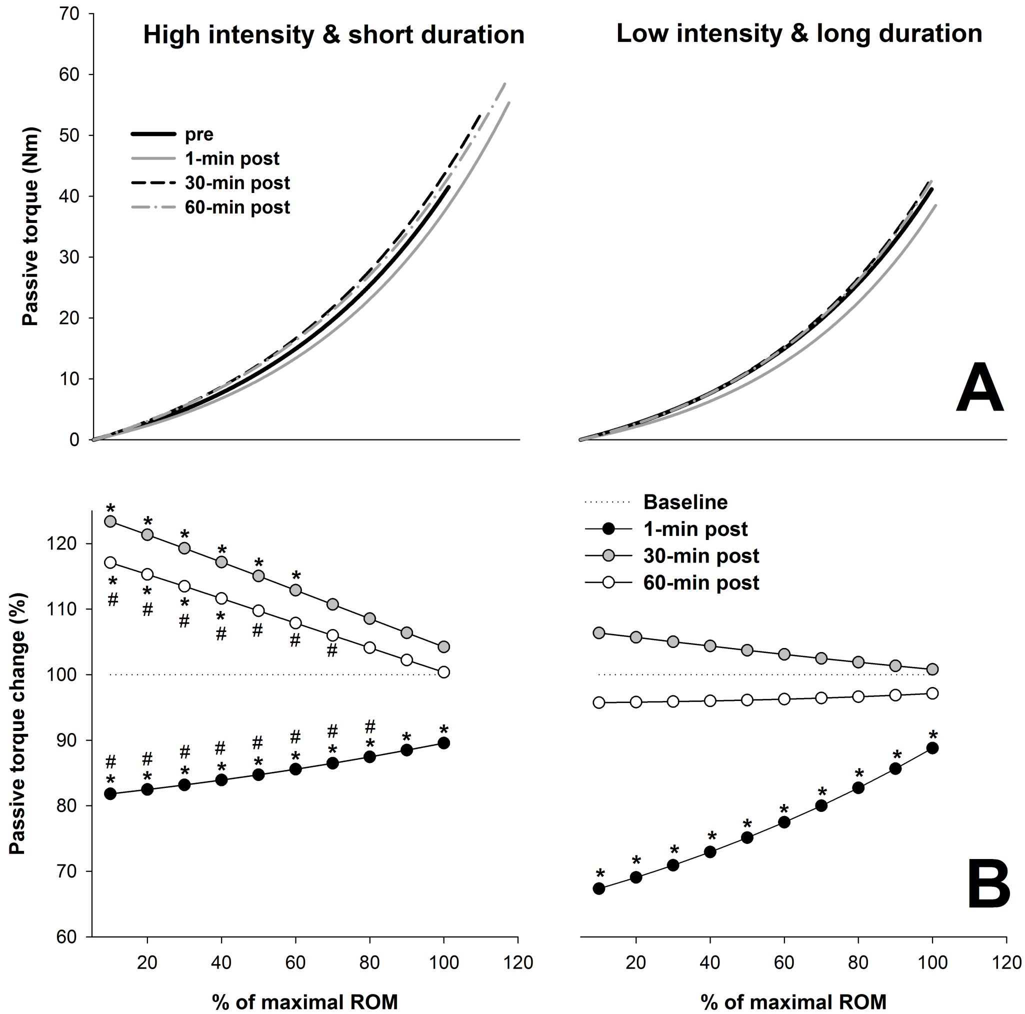

Figure 30. Typical example for one participant of (A) a passive torque-ROM curve and (B) the percentual changes in passive torque before (i.e., baseline) and at 1, 30, and 60 minutes after the high-intensity and short-duration stretching protocol (HISD) and the low-intensity and long-duration protocol (LILD).

Note: 1) X-axis is the % of maximal ROM obtained in the first repetition (i.e. pre condition); 2) The error bars are not shown in Figure 2-B for better image observation.

* – Statistical difference from baseline (*P*<0.05).

– Statistical difference between protocols for the same percentage of ROM (p<0.05).

The maximal ROM and peak torque before and at 1, 30 and 60 min after the stretching for both protocols are depicted in Figure 31. A significant interaction (protocol × time) was observed for maximal ROM (p=0.005) and peak torque (p=0.009). A significant effect for time was found for maximal ROM (p=0.00001) and peak torque (p=0.00001). A significant effect for protocol was seen for maximal ROM (p=0.003) and peak torque (p=0.025).

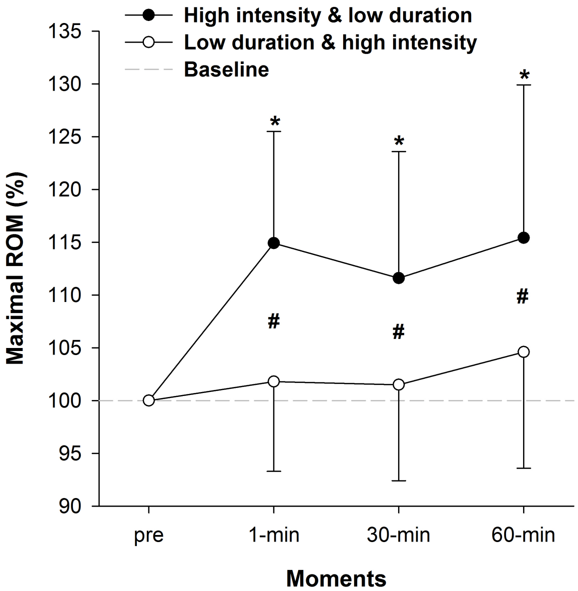

Figure 31. Maximal range of motion (left) and peak torque (right) before and at 1, 30 and 60 minutes after the two stretching protocols.

Values are normalized to the baseline (i.e. first repetition) condition.
* – Statistical difference from baseline condition ($p<0.05$).
# – Statistical difference between protocols ($p<0.05$).

No significant effect for time ($p>0.09$) or protocol ($p>0.62$) was found on EMG in both muscles. The muscle activity of both ST and VM muscles was lower than 3% during the HISD stretching, and lower than 1.5% during the LILD protocol. At the testing moments before and after stretching (1, 30, and 60-min), the average EMG was below 1.6% in both muscles.

Discussion

The present study compared two stretching protocols with a reverse proportion of stretch intensity and duration (i.e., high intensity with short duration vs. low intensity with long duration). The EMG was lower than 3% for both protocols; thus, we assumed that passive torque measurements were not affected by muscle activity. Also, there was no significant difference in passive torque and maximal ROM of the first repetition between protocols, suggesting the participants were in the same condition at the beginning of the stretching interventions. The intensity achieved by the HISD was higher than the initial (i.e., compared to the first repetition), and the stretch duration was lower due to the lower number of 90-s repetitions. Consequently, stretch intensity (peak torque: 109.2±10.4% vs 50%; ROM: 107.3±7.6% vs 71.9±4.2%) and duration (243.5±69.5 vs. 900-s) were applied in an inverse mode among protocols.

The stretching protocols produced different responses in torque and angle one hour after stretching. An increase of peak torque and maximal ROM was observed only in HISD, for all moments tested after stretch (Figure 3). No significant increases were observed for LILD, despite the longer stretch duration. This indicates that in order to increase the maximum tolerable torque and ROM, the stretch intensity should be maximal, even with a lower stretch duration. Such result contradicts the premise of Jacobs and Sciascia (2011), who advocate the view that stretch duration and intensity are inversely related. On the other hand, the present results support the studies that suggest a higher stretching intensity for increasing the maximal ROM (Light et al., 1984; McClure et al., 1994; Usuba et al., 2007). In addition, another observation was that the peak torque-angle outcomes with the HISD protocol still increased at 30 and 60 min after stretching despite the fact that passive torque returned to baseline values. This result is in accordance with previous studies (Mizuno et al., 2011, 2013).

Regarding the effects of stretching on the torque-angle curve, a different response was observed post-stretching between protocols. Both stretching protocols decreased the passive torque 1 min after stretching; however, a greater torque decline was observed for LILD in the initial portion of the knee ROM. This suggests that stretch duration provides a more acute torque decline than stretching intensity. Again, this result contradicts again Jacobs and Sciascia's (2011) premise. Moreover, at the 30-min post-stretching, an increase of passive torque above the baseline was observed for the HISD in the initial torque-angle curve range, but not for LILD. The increased passive torque for HISD was still observed 60 min after stretching, and was significantly different from the LILD passive torque. These results were unexpected, and to our knowledge this is the first study to report a passive torque increase after high-intensity stretching. Mizuno et al. (2013) found a similar response for stiffness (i.e., slope of the torque-angle curve) 30 min after a static stretching of the calf muscles with five repetitions of 1 min each (see Figure 4 of Mizuno et al. 2013 study). However, they did not report a statistical difference. This probably occurred because they used a rest interval stretching protocol, and thus produced a lower stretching intensity. We have previously observed (see study 5, page 101) that non-resting between repetitions induces a higher ROM and peak torque than static stretching with rest between repetitions. In the present study, no resting was performed between the 90-s repetitions for the HISD, and consequently a higher intensity was achieved. Thus, we suspect that the higher intensity causes the passive torque increase.

The mechanism underlying the passive torque increase 30-60 minutes after the HISD is unknown. We speculate four possible situations. Recently, Schleip et al. (2012) reported an increase of water content in mice lumbodorsal fascia above baseline values, after high-intensity stretching. Thus, it is possible that a high-intensity stretch might induce overcompensation in the water content of the connective tissue being stretched, since the extracellular matrix is largely responsible for the viscoelastic characteristics of connective tissue (Lu, Parker, & Wang, 2006). Thus, joint passive torque might have increased as a consequence. A second hypothesis is related to muscle damage. Previous studies suggest that static stretching induces more relative deformation of muscle components than the tendon among repetitions (Abbelaneda, Guissard, & Duchateau, 2007; Abellaneda, Guissard, & Duchateau, 2009; Nakamura et al., 2013). Thus, a higher-intensity stretch might have induced some damage in the muscle component. Whitehead and colleagues (2003) found that passive tension of the cat's gastrocnemius muscle increased above baseline values 40 min after eccentric contractions. We do not know if NRI protocol have overstretched muscle fibers and consequently produced structural damage to the fibers' membranes. If such a situation occurred, it might have increased intracellular calcium concentration and thus have increased tension in the muscle. The third hypothesis can be related to an increase of muscle tone as a consequence of a higher reflex activity; however, no differences were observed between EMG of the muscles tested between testing moments. Hence, we admit that neural factors had no influence of the passive torque increase. Finally, the fourth hypothesis may be related to structural and mechanical properties changes in the muscle-tendon complex; however, the results of previous studies on the immediate acute changes in the tendon stiffness, fascicle length and pennation angle after the static stretch are controversial (Kay & Blazevich, 2009; Mizuno et al., 2011; Morse et al., 2008).

In conclusion, the timecourse of the joint torque-angle response after stretching differs between a high-intensity and short-duration stretch and a low-intensity and long-duration stretch. The increase in peak torque and maximal angle was observed for 60 minutes after stretching for the higher-intensity stretch. On the other hand, the protocol with higher duration induced a more acute decrease in passive torque. Thus, stretch intensity was seen as more important for ROM increase, and duration appears to be more important for acute passive torque decline. In addition, the stretching with the highest intensity increased the passive torque above the baseline 30 and 60 minutes after the stretch. Future studies should investigate the long-term effects of stretching with different intensities and durations.

Study 7 – Muscle and joint physiological responses to static stretching at different intensities.

Design

A quasi-experimental design was used to observe the effects of three stretching protocols with three different intensities. Stretch intensity was considered as a percentage of maximal ROM. The participants were familiarized with the experimental setup and visited the laboratory on three occasions (Figure 32). Pre and post stretching tests were performed for the purposes of the study.

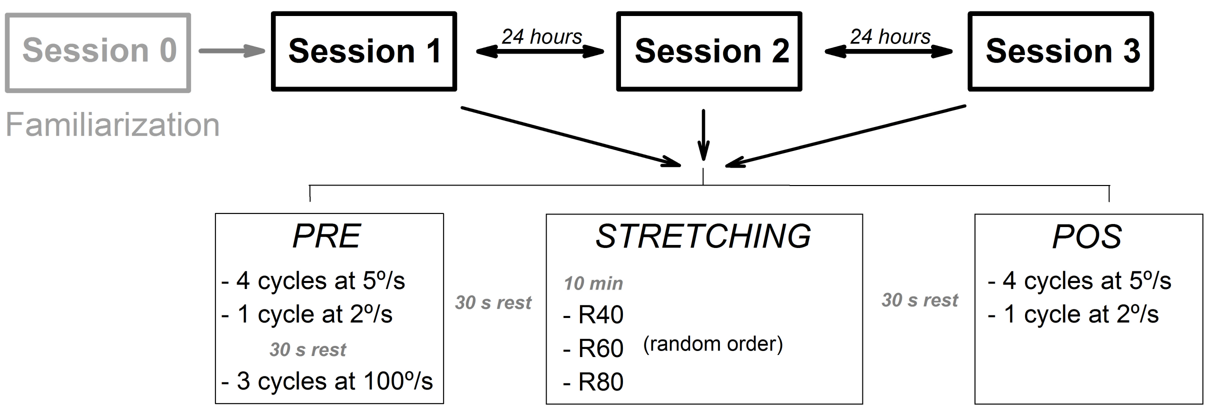

Figure 32. Study 7 design.

Protocol

Three 10-min static stretching sessions were performed on different days with a random and balanced order, for three different stretch intensities. For each session, upon arrival of participants to the laboratory, skin was prepared for EMG, and then participants laid prone with the knee fully extended. The maximal dorsiflexion passive ROM was determined in the first session by manually moving the platform fixed to the foot. Then, four dorsi- to plantarflexion cycles (from -40° in plantar flexion position to 80% of maximal dorsiflexion ROM) at 5 °/s were performed for conditioning, followed by a fifth cycle at 2 °/s to assess the inter-day reliability of measurements, and to compare to a cycle post stretching. After, three rapid stretches at 100 °/s were performed for SR testing habituation, and EMG was visually inspected to assure that no muscle activity would occur during the stretching maneuver. A 10-min static stretching was then imposed 30 s after by displacing the ankle at 100 °/s from the -40° plantar flexion position to the target dorsiflexion position. Depending on the session, the target dorsiflexion position was set at 40%, 60% and 80% of the previously determined maximal ROM (R40, R60 and R80). Thirty seconds after the stretching, four cycles at 5 °/s and a fifth cycle at 2 °/s were performed to determine the stretching post effects. In the end of the session, three maximal voluntary plantarflexors isometric contractions were performed to normalize the EMG values.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19.0 (IBM Corporation, New York, USA). Normal distribution was checked for all data variables using Shapiro-wilk test. Inter-day assessment reliability was determined using intraclass coefficient correlation (ICC) at a 95% confident interval (CI) and standard error of measurement (SEM) for passive torque and SSI measurements at the resting plantarflexion position and 65%, 70%, and 80% of maximal dorsiflexion ROM, by comparing the pre-conditioning cycle at 2°/s between sessions. The inter-examiner digitizing reliability for fascicle length manual measurements and the inter-examiner reliability for determination of the angle corresponded to the slack length were determined using the ICC and SEM. Automatic routine tracking digitizing reliability (i.e. by processing a pre-stretching cycle of each participant two times), tracking repeatability (i.e. processing two successive pre-stretching cycles for each participant), and inter-day assessment reliability (i.e. comparison between the pre-cycles of each protocol for each participant) were determined at resting plantarflexion position for absolute FL values, and at 65, 70, and 75% of dorsiflexion ROM for both absolute and relative (i.e to resting ankle position value) values using the ICC and SEM. For the SR analysis, four two-way ANOVAs [protocol (R40, R60, R80) x time (SR start, SR end)] were performed for each variable (passive torque, shear elastic modulus, fascicle length, and VAS score). Then, eight one-way ANOVAs [protocol (R40, R60, R80)] were performed on absolute and relative SR values (i.e. normalized to the maximum value during SR) for each variable. When a significant interaction was found between factors, post hoc one-way ANOVA for protocols and paired t-tests for time were performed to detect individual differences. For the analysis of stretching effects, a two-way repeated measures ANOVA [protocol (R40, R60, R80) × time (pre, post)] was performed for passive torque, shear elastic modulus, fascicle length, and muscle stiffness for the angles corresponding to 65, 70 and 75% of maximal ROM, and for average EMG during the ankle cycles. When a significant effect was observed, post hoc Bonferroni analysis was performed. Pearson correlation coefficient (*r*) and ICC were used to determine the relation between the size of stretching pre to post effects and SR magnitude response on joint torque and muscle shear elastic modulus. Statistical significance was set to $p<0.05$.

Results

Reliability. The inter-day assessment reliability during the stretching was high for SSI (65%ROM: ICC = 0.79 (0.55 - 0.93), SEM = 5.4 kPa; 70%ROM: ICC = 0.77 (0.51 - 0.92), SEM = 9.2 kPa; 75%ROM: ICC = 0.68 (0.36 – 0.88), SEM = 14.78 kPa), and high to very high for torque (65%ROM: ICC = 0.88 (0.72-0.96), SEM = 0.72 Nm; 70%ROM: ICC = 0.94 (0.84-0.98), SEM = 0.88 Nm; 75%ROM: ICC = 0.95 (0.89-0.98), SEM = 1.05 Nm). The inter-day assessment reliability of resting SSI was very low [ICC=0.07 (-0.28-0.50), SEM=2.78 kPa], but resting SSI repeatability was very high [ICC=1.00 (1.00-1.00), SEM=0.1 kPa]. The procedure to determine the gastrocnemius slack length showed a very high reliability when determined by different examiners (ICC=0.92 [0.82-0.97], SEM= 2.75°). The inter-digitizing reliability for manual fascicle tracking was high (ICC=0.93 [0.82-0.98], SEM=2.9mm) between the three examiners. A high reliability was observed for automatic routine tracking digitizing (65%ROM: ICC = 0.94 (0.83 - 0.98), SEM = 0.20 mm; 70%ROM: ICC = 0.96 (0.89 - 0.99), SEM = 0.15 mm; 75%ROM: ICC = 0.94 (0.82 – 0.98), SEM = 0.22 mm) and tracking repeatability (65%ROM: ICC = 0.88 (0.65 - 0.97), SEM = 0.38 mm; 70%ROM: ICC = 0.94 (0.80 - 0.98), SEM = 0.31 mm; 75%ROM: ICC = 0.96 (0.87 – 0.99), SEM = 0.26 mm), but low to moderate for inter-day assessment reliability (resting position: ICC = 0.54 (0.16 - 0.81), SEM = 0.62 mm; 65%ROM: ICC = 0.46 (0.07 - 0.77), SEM = 0.93 mm; 70%ROM: ICC = 0.50 (0.12 - 0.79), SEM = 0.96 mm; 75%ROM: ICC = 0.50 (0.12 – 0.79), SEM = 1.02 mm).

Medial gastrocnemius slack length. The pooled mean of the ankle angle corresponded to the slack length was -9.3±8.1° of plantarflexion.

EMG. All muscles EMG were bellow 1% of MVC during the SR measurements.

Stress relaxation. An example of torque and SSI relaxation during stretch at the three different ankle angles for one participant is shown in **Figure 33**.

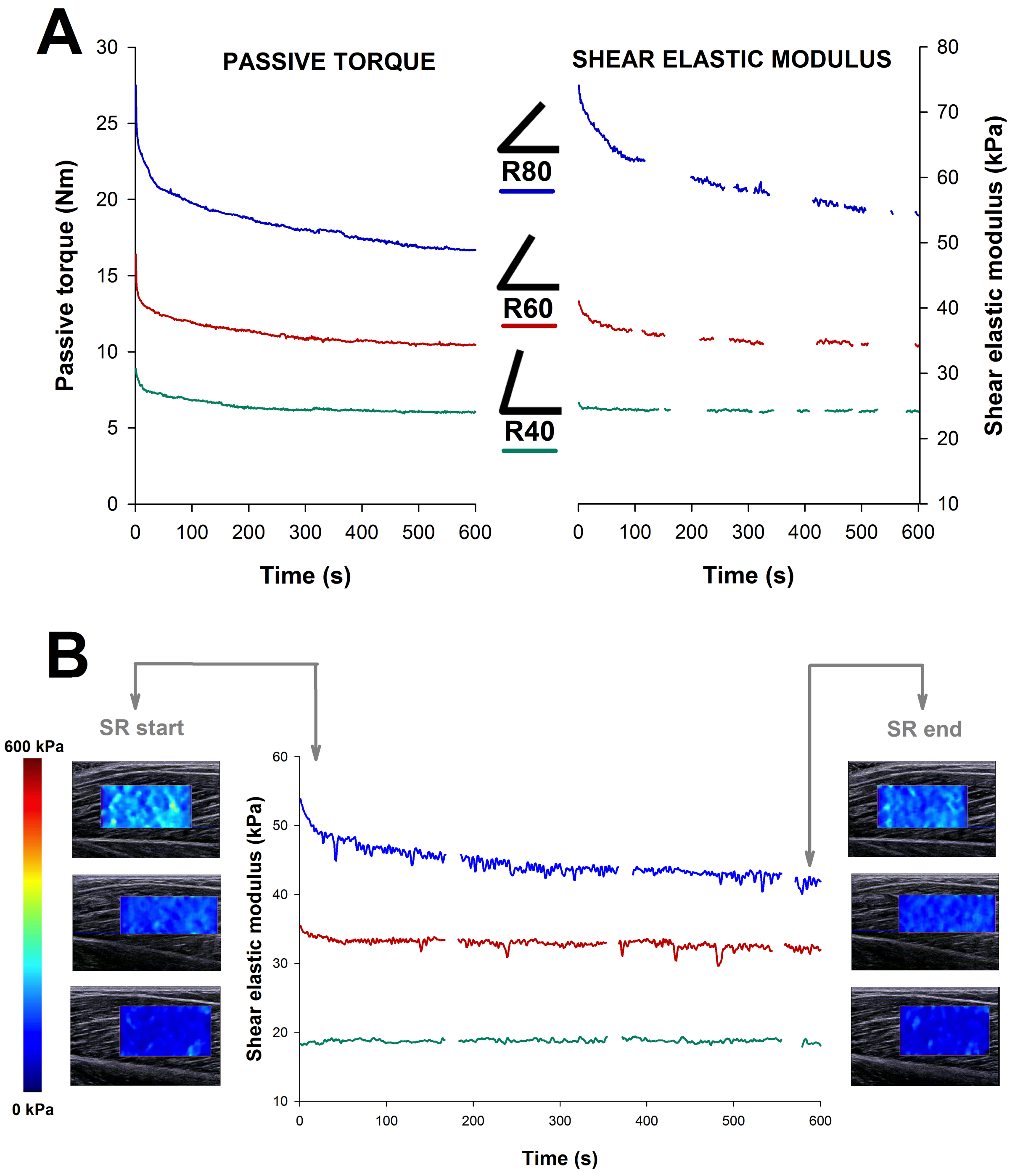

Figure 33. A) Average values for ankle passive torque (left) and shear elastic elastic modulus (right) relaxation during a 10-min static stretch at three different intensities; B) Shear elastic elastic modulus during a 10-min static stretch at three different intensities for one participant (#6).
R80 – Stretch at 80% (blue) of maximal range of motion (ROM); R60 - Stretch at 60% (red) of ROM; R40 - Stretch at 40% (gree) of ROM.

A main effect of time was found for torque (p=0.0001) and shear elastic modulus (p=0.0001), while this was not the case for fascicle length (p=0.12). In addition, a significant protocols × time interaction was found for passive torque (p=0.014), shear elastic modulus (p=0.00001), while this was not significant for fascicle length (p=0.93). A decrease in passive torque was observed for all protocols (R80=-7.0±5.2 Nm, p=0.002; R60=-3.4±1.8 Nm, p=0.0002; and R40=-2.1±0.8 Nm, p=0.00001). For SSI, a significant decrease in shear elastic modulus was found for R80 (-19.1±9.1 kPa, p=0.00001) and R60 (-6.8±4.9 kPa p=0.002), but not for R40 (-1.3±1.8 kPa p=0.06).

The statistical analysis concerning absolute changes showed main effects of protocol for torque (p=0.003, Figure 34-A) and shear elastic modulus (p=0.00001, Figure 34-B), but not for fascicle length (p=0.372, Figure 34-C). Concerning relative changes, main effects of protocols were found for the shear elastic modulus (p=0.00001, Figure 34-B), but not for passive torque (p=0.444, Figure 2-A) and fascicle length (p=0.439, Figure 34-C).

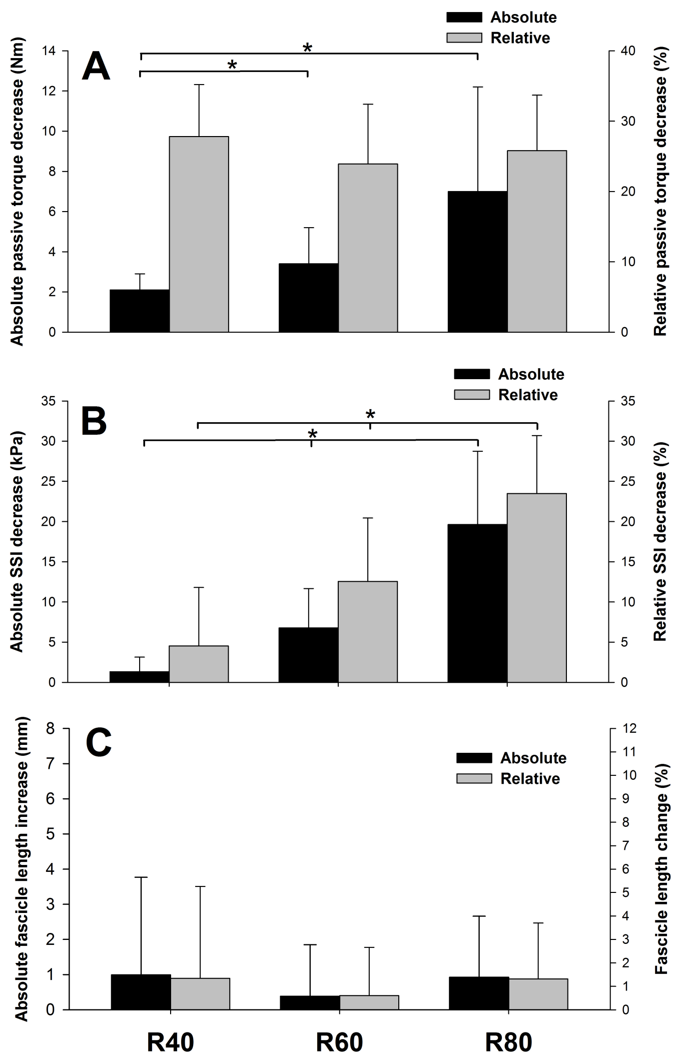

Figure 34. Absolute and relative (normalized to peak value) of stress relaxation for passive torque (A) and shear elastic modulus (B), fascicle length change (C), and the VAS score (D).
* Statistical difference at p<0.05.

Stretching effects. The average values of the shear elastic modulus and passive torque responses for all participants before and after the three different stretching protocols are depicted in **Figure 35**.

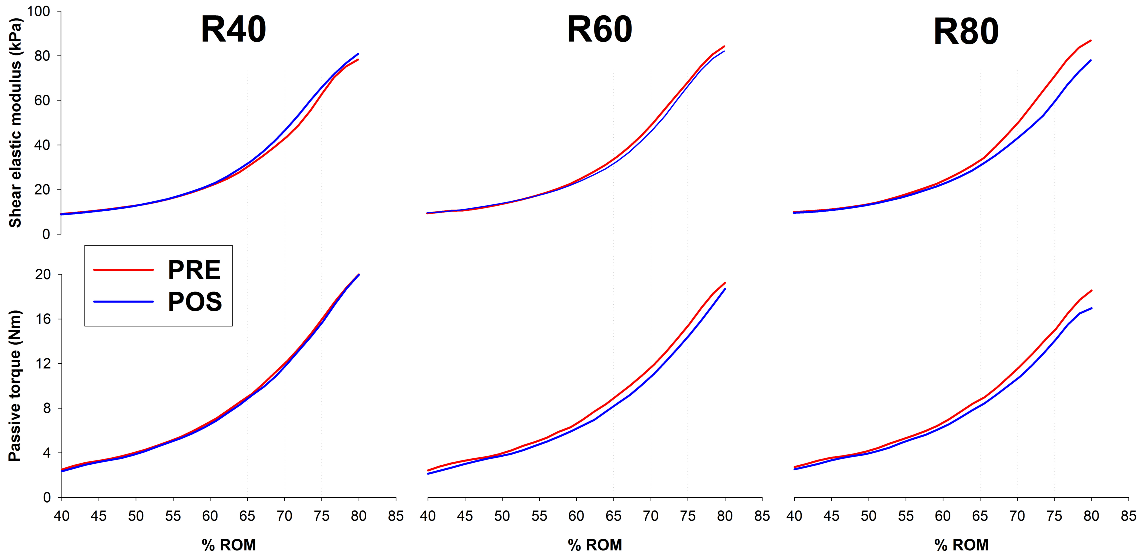

Figure 35. Average response of shear elastic modulus and passive torque before and after the stretching intervention for the different intensities.

The changes in shear elastic modulus and passive torque after the three stretching protocols is showed in **Figure 36**. For the passive torque, a significant effect was observed for: time at 65% ($p=0.01$), 70% ($p=0.0001$), and 75% of ROM ($p=0.02$); protocol at 70% of ROM ($p=0.03$); protocol × time at 70% of ROM ($p=0.03$). No effect was found for shear elastic modulus at 65% and 70% of ROM, but an effect was observed at 75% of ROM for protocol × time ($p=0.04$), protocol (0.03), time ($p=0.04$).

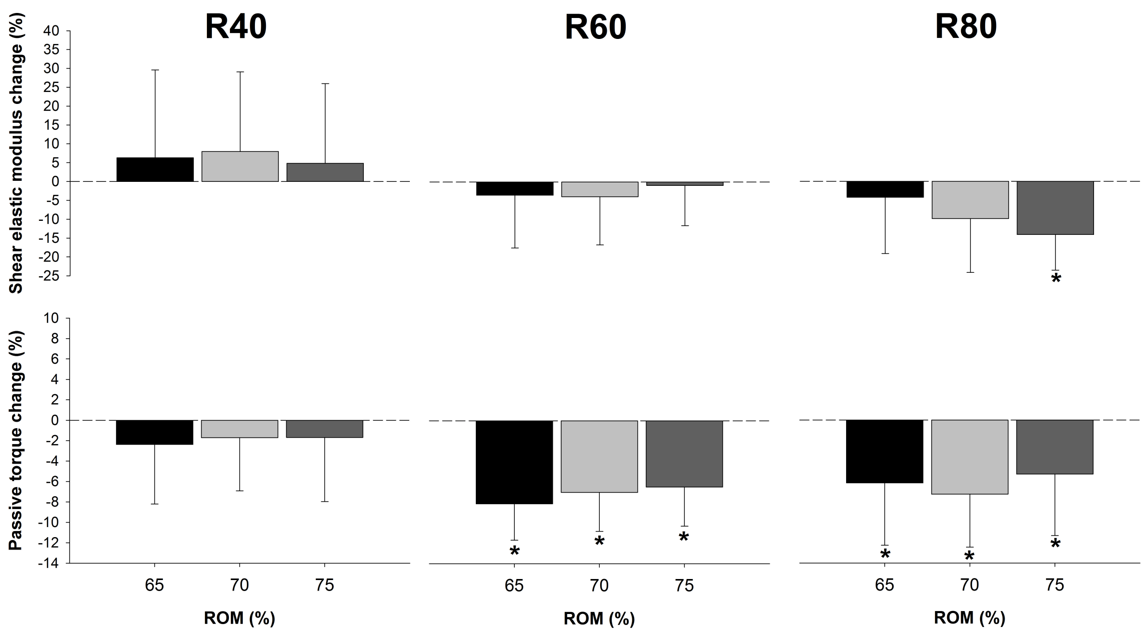

Figure 36. Passive torque and shear elastic modulus changes after the three static stretching protocols at three distinct angles.

* – Statistical different from the baseline (p<0.05).

ROM – maximal range of motion (ROM); R40 – 40% of ROM; R60 – 60% of ROM; R80 – 80% of ROM

For the three angles tested, no significant effect was observed for protocol (p=0.23-0.24), time (p=0.31-0.67), or protocol × time (p=0.23-0.59) for the fascicle length after stretching. Similar results were obtained for muscle stiffness with no significant effect for protocol (p=0.48-0.69), time (p=0-06-0.12), or protocol × time (p=0.13-0.40).

Stress relaxation vs. stretching effects. No significant correlation was observed between the size of post stretching effects on passive torque for stretching protocols of R40 (ICC = -0.26 [-0.69-0.30], r = -0.24), R60 (ICC = 0.15 [-0.40-0.62], r =0.19), and R80 (ICC = 0.29 [-0.28-0.70], r = 0.26), and on shear elastic modulus for R40 (ICC = 0.18 [-0.37-0.64], r =0.41), R60 (ICC = 0.09 [-0.46-0.58], r = 0.09), and R80 (ICC = 0.14 [-0.42-0.61], r = 0.15).

Discussion

Joint torque-angle, muscle shear elastic, and muscle fascicles length were assessed before, during and after three stretching protocols with different stretching intensities. The main findings of this study were: i) muscle SR was seen to be independent on its length, and fascicles length do not change during the SR; ii) the stretching effect on joint torque and muscle shear elastic modulus depend on stretching intensity;

iii) the joint torque response during and after stretching do not reflect changes in muscle passive tension and stiffness; iv) the magnitude of stretching effects on torque and muscle shear elastic modulus is unrelated to the size of SR.

Stress relaxation was measured in human ankle musculo-articular complex (i.e., torque) and medial gastrocnemius (i.e., passive tension) in vivo at three different ankle angles. A low EMG activity (<1%) was observed during SR for all subjects. Thus, we assume that basal muscle tone did not affected SR measurements (Gajdosik, 2006). In respect to reliability assessment, we found acceptable results for all variables. To our knowledge, the present study is first to report inter-day reliability of SSI measurement during passive muscle stretching. These reliability results are similar to those of the Maïsetti et al. (2012) study.

It is often assumed that muscle relaxation occurs at a length beyond the slack length (Abbott & Lowy, 1956). It is also reported that when the muscle *in vitro* is stretched slightly above the slack length, the increased stress decays to the resting values (Abbott & Lowy, 1956). When the stretch is higher, the force decay does not fall to resting values (Abbott & Lowy, 1956). In the present study we have stretched the plantar flexors muscles in three muscle lengths beyond the muscle slack length. Using SSI measurements, a decrease was observed in SSI for only the two highest intensities (R60 and R80), not for R40. It is possible that the muscle SR in vivo has a different pattern response compared to an in vitro condition, because muscle tissue is surrounded by connective tissue and thus a force transmission may occur between these two tissues.

An interesting finding was that absolute and relative muscle passive tension relaxation estimated using SSI was shown to be dependent of muscle length. This result contradicts the previous study of Tian et al. (2010). It was concluded that ankle relaxation (i.e., torque decrease in a static and stressed position) was affected minimally by changing gastrocnemius muscle–tendon unit length. In our study, we observed that the absolute and relative muscle SSI relaxation was higher for a greater muscle length. Hence, muscle SR is dependent on its length. The discrepancy between both studies might be explained by the different protocols used. Indeed, Tian et al. mostly manipulated the knee angle, while the ankle angle was changed in the present study. Due to interactions between mono and bi-articular structures (Bojsen-Møller, Schwartz, Kalliokoski, Finni, & Magnusson, 2010; Tian, Herbert, Hoang, Gandevia, & Bilston, 2012), these protocols could have different effects on the SR.

Another unexpected result was that the magnitude of torque relaxation was not similar to muscle relaxation, for both SR relative values and for different stretch intensities. This means that the contribution of gastrocnemius relaxation is not proportional to the ankle torque relaxation, and thus indicates that other anatomic structures might have different SR responses and that depends on stretch intensity.

The present results also do not support the Nakamura et al. (2013) study that observed a MTJ displacement of approximately 4.5mm during a 5-min static stretch. Based on these results, we would expect to observe an increase of about 4.7 mm in fascicles length in the current study (i.e., considering the average of 18° pennation angle and a fascicle length of 69 mm observed in our study). However, we observed no changes in fascicle length during the 10-min static stretch. A possible reason for these different results might be attributed to the aponeurosis. However, another reason could be related to the different methodological procedure used in our study and Nakamura study (i.e., blinded condition). Thus, the results of the present study suggest that the SR is similar in fascicles and tendinous structures.

Regarding the immediate effects after static stretching, the previous studies have suggested that muscle passive stiffness decreases after static stretching based on passive torque measurements (Gajdosik, 2001; Magnusson et al., 1996; McHugh & Cosgrave, 2010; Mizuno, Matsumoto, & Umemura, 2013). However, because of methodological barriers, it was never investigated the factual effects of stretching on the properties of the muscle. Since in the last years the imaging technology has advanced and allowed to start investigating the passive properties of the muscle *in vivo* (Gennisson et al., 2010; Koo et al., 2013), only now it was possible to realize the relationship between the joint passive torque and the muscle passive tension response to stretching. The results of the present study do not support the conclusions of previous studies based on torque measurements. For instance: the R40 stretching did not induced changes in torque and SSI measures; the R60 stretching decreased the torque but not the SSI; the R80 stretching decreased the torque, and only decreased the SSI measurement at 75% of the maximal ROM. This suggests that the passive torque response do not reflect total changes in muscle passive tension. Also, it suggests that both effects on joint passive torque and muscle passive tension induced by static stretching appear to be dependent of the stretching intensity. It was only observed a significant decrease of muscle passive tension at 75% of maximal ROM when a R80 stretching was performed. The previous studies examining the effects of stretching at different intensities have not examined the effects on joint torque or either muscle passive tension (Walter et al., 1996). Thus we think that this is the first study reporting these effects. In addition, the present results also do not support the studies that suggest a low stretching intensity for a higher passive torque decrease (Light et al., 1984; Usuba et al., 2007). The passive torque decrease was higher for the R80 R60 compared to R40, with no significant differences observed between R40 and R60. Also, the muscle passive tension was only decreased at 75% of maximal ROM for R80, and thus reinforcing the stretching effects dependence on its intensity. It has been suggested that longer stretching duration induces higher torque decrease, however the stretching intensity also might play a role in torque decrement.

In respect to the effects of stretching on resting SSI measurements, we have observed a very low inter-day reliability, but a good repeatability within the same session. Thus we were able to compare the effects within each protocol before and after stretching. We think that the low inter-day reliability is due to the use of the cast to fix the probe to the muscle belly. The previous studies inter-day reliability for resting SSI measurements on medial gastrocnemius have reported a high reliability but assessing manually and performing minimal pressure on the skin (Akagi & Takahashi, 2013; Lacourpaille et al., 2012). It has been shown previously that the mechanical pressure performed by the probe on the skin affects the muscle SSI measurements (Kot, Zhang, Lee, Leung, & Fu, 2012). Consequently, the use of a cast to fix the probe may have affected the SSI resting measurements due to different probe pressures on the skin. However, it has observed no changes on muscle SSI at rest. This result contradicts the study of Akagi & Takahashi et al. (2013). The reasons for different results might be related to the different assessment procedures. In the present study the probe was placed on the muscle mid-belly according to the fascicles direction, and in the Akagi & Takahashi et al. (2013) study the probe was placed transversely to the muscle. The previous methodological studies suggest that the probe should be placed according to the fascicles orientation, so the measurements could have better correlation to the muscle passive tension and stiffness. Also, the intensity of the stretching in the present study was submaximal, and in Akagi & Takahashi et al. (2013) study the stretching was maximal ROM whereas participants felt “discomfort or pain”. Since it was observed that the effect on muscle shear elastic modulus depends on the stretching intensity, it is possible that this also explains different results.

Another observation of this study was that the muscle stiffness was unchanged after static stretching for the protocols. The previous studies examining the effects of stretching on the muscle-tendon passive properties have observed different results (Herda et al., 2011; Kato, Kanehisa, Fukunaga, & Kawakami, 2010; Kay & Blazevich, 2009; Kubo et al., 2002; Morse et al., 2008). For instance, the tendon stiffness is reported to be decreased (Kato et al., 2010) or not be affected (Kay & Blazevich, 2009; Kubo et al., 2002; Morse et al., 2008). The muscle stiffness is stated to decrease (Kay & Blazevich, 2009; Morse et al., 2008), or not be affected (Kato et al., 2010). The muscle-tendon unit stiffness is reported to decrease (Morse et al., 2008) or not change (Herda et al., 2011). However, it should be considered that the muscle stiffness measurements have been determined under certain assumptions that have not been confirmed. For instance, Kay & Blazevich (2009) calculated muscle stiffness through the relation between the muscle fascicles length and the joint passive torque assuming that the joint torque would reflect the muscle passive tension. Also, Kato et al. (2010) calculated muscle stiffness as a muscle fascicles length divided by the muscle-tendon unit length, and this is known as deformation (Baumgart, 2000). However, the stiffness measurements implies that length and passive tension from the tissue is known (Baumgart, 2000). In the present study the passive tension was assessed *in vivo* using the supersonic shear wave elastography, and length was determined using ultrasonography assessment in B-mode. In the other hand, it should be considered that the stretching intensity was submaximal, and perhaps with a more intense stretch we would observe a decrease of muscle stiffness.

Moreover, previous have assumed that the degree of the stretching effects on joint passive torque and muscle stiffness depend on the size of the SR that occurs during the static stretching (Cabido et al., 2014; Herda et al., 2011). Consequently a higher stretching duration and intensity has been advocated to increase the magnitude of the stretching effects as a consequence of a higher stress relaxation. However, the results of the present study did not showed a relation between the magnitude of the stretching effects and the size of stress relation in both muscle shear elastic modulus and joint passive torque. This means that other factors not examined should be responsible for the magnitude of stretching effects on muscle and joint than the size of SR.

In conclusion, it was observed in this study that the medial gastrocnemius relaxation appears to be independent on muscle-tendon unit length; muscle fascicles length do not change during a static stretching; the effects on the joint passive torque after static stretching do not reflect changes in the muscle passive tension, fascicle length, and muscle stiffness; the stretching effects on joint and muscle depend on its intensity; and submaximal stretching intensities do not affect the muscle stiffness. Future studies should investigate the effects of maximal and supramaximal intensities on muscle passive properties.

Study 8 – Muscle response to high intensity stretching.

Design

An experimental study was conducted to extend the conclusions of the study 6 (page 109), and thus to determine if the joint passive torque increase 30 minutes after a non-rest interval stretching protocol (NRI) was due to a muscle response (Figure 37). The participants were familiarized with the experimental setup and visited the laboratory on two occasions to perform a NRI protocol with and without MVC tests after the stretching.

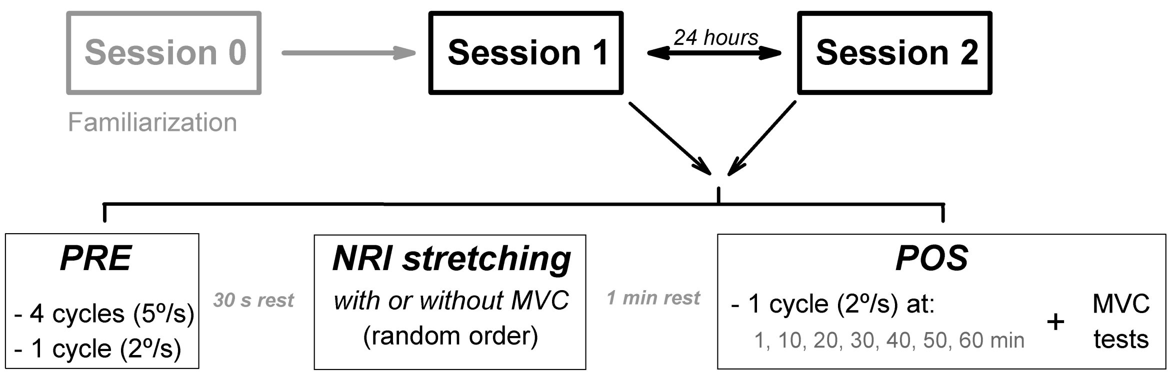

Figure 37. Study 8 design.

Protocol

A NRI stretching protocol targeting the ankle plantarflexors muscles was performed in each session (Figure 8, page 44). In one session (MVC), a maximal voluntary isometric contraction (MVIC) was performed 5-min before and 1, 10, 20, 30, 40, 50, and 60-min after the stretching protocol. In the other session (no-MVC), a MVIC test was performed only 5-min before and 60-min after the stretching protocol. The MVIC consisted in three maximal voluntary contractions of plantarflexors with the foot in a neutral position (i.e. 0°) by resting 15 s between trials. Sessions were performed in a balanced order. Sessions began with the MVIC testing, followed by a 4 plantar-/dorsiflexion cycles at 5°/s and fifth cycle at 2°/s for condition purposes. Cycles were performed from the 40° of plantarflexion position to the 20° of dorsiflexion. For testing post-stretching effects, a cycle at 2°/s was performed 1, 10, 20, 30, 40, 50, and 60 minutes after the stretching protocol. In the MVC session, the stretching cycle was always performed before the MVIC tests. In the time between cycles and MVIC tests, the ankle rested in a 20° plantarflexion ankle position, so plantarflexors muscles could be in a slack length (Hug et al., 2013). The

NRI consisted in performing the maximal number of stretching repetitions without resting between repetitions, until the point in which participants could not stretch further without feeling pain (Figure 13-B). Each repetition lasted 90 seconds in the stretching position, and the stretching maneuver was performed with an angular velocity of 2°/s. The same number of NRI repetitions and the ankle angles performed in the first session was reproduced in the second session, so the stretching intensity and duration could be the same. A NRI protocol was used because it was previously shown that it induces a higher stretching intensity compared to a conventional rest interval protocol [see study 2 (page 67) and study 5 (page 101)].

Statistical analysis

All data was analyzed using IBM SPSS Statistics 19.0 (IBM Corporation, New York, USA). The data is presented as normalized to baseline (pre) values (mean ± SD). Normal distribution was confirmed using Shapiro-wilk test. Both SSI and torque values the angles corresponding to the 90% of the cycle range of motion (ROM) were calculated for comparisons between the different testing moments. A t-test was used to compare the SSI and the torque of the pre-stretching cycle, and the pre MVIC test between the two sessions, to assure that participants were in the same condition at the beginning of the stretching protocol. A two-way ANOVA [protocol (MVC, no-MVC) × time (pre, 1min, 10min, 20min, 30min, 40min, 50min, 60min)] was performed for comparisons of SSI, SSI ED, SSI DC, passive torque, torque ED and torque DC values. Two-way repeated measures ANOVA [protocol (MVC, no-MVC) × time (pre, 60min)] was performed for MVIC. When the sphericity assumption in repeated measures ANOVAs was violated (Mauchly''s test), a Geisser-Greenhouse correction was used. When an interaction for protocols x time was observed a one-way repeated measures followed by contrasts (pre vs. post stretching time testing) was performed for time moments, and paired t-tests were performed to compare protocols for each testing time. A one-way repeated measure followed by contrasts was performed for MVIC in the MVC session. Statistical significance was set at $p<0.05$.

Results

No differences were found for passive torque ($p=0.29$) and SSI ($p=0.21$) between the pre cycles of the two stretching sessions, and for MVIC ($p=0.11$) values. The participants performed the same number of stretching repetitions in both sessions (2NRI, n=2; 3NRI, n=3, 4NRI, n=2, and 5NRI, n=4). A typical example for one participant of ankle passive torque, SSI, and MVC response in all testing moments can be observed in Figure 38.

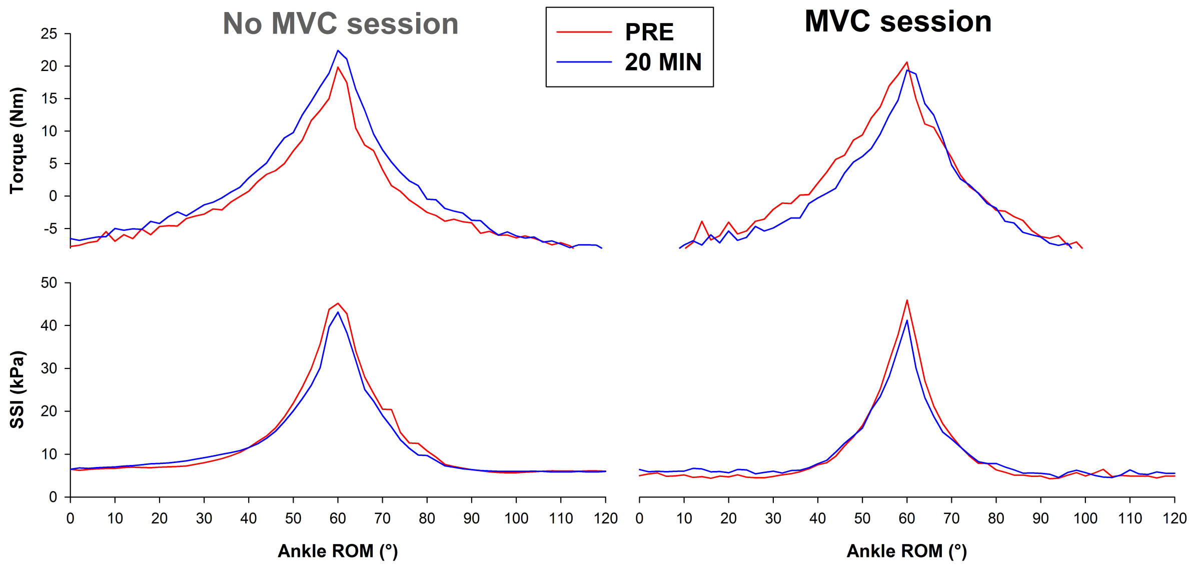

Figure 38. Typical example for one participant (#5) load/unload cycle response of passive torque and gastrocnemius shear elastic modulus (SSI) response before and 20 minutes after the non-rest interval stretching protocol, in both MVC and no-MVC sessions. In the x-axis, the 0, 60, and 120 values corresponds to 40° of plantarflexion, 20° of dorsiflexion, and 40° of plantarflexion, respectively. Raw data values are presented for every 2°.

Passive torque. The passive torque, torque ED, and torque DC results are depicted in Figure 39. A significant effect on protocol × time ($p=0.017$), time ($p=0.0001$) and protocol ($p=0.027$) for passive torque was observed. The passive torque decreased at 1-min after stretching in both MVC (-10.1±6.25%, $p=0.0001$) and no-MVC (-7.5±8.4%, $p=0.015$) sessions. For the no-MVC session, the torque increased above baseline at 20-min (+7.5±13.9%, $p=0.01$) and 30-min (+6.3±9.3%, $p=0.049$), and returned to baseline 40-min ($p=0.23$), 50-min ($p=0.44$) and 60-min ($p=0.67$) after stretching. In the MVC session, torque decreased above baseline at 1-min, 10-min (-6.3±8.2%, $p=0.03$), 20-min (-8.0±9.2%, $p=0.017$), and 60-min (-9.2±12.4%, $p=0.034$) minutes after the stretching. Torque was significantly different between sessions at 20-min ($p=0.013$) and 30-min (+6.3±9.3% vs -5.6±13.2%, $p=0.017$). A significant effect for time on torque ED ($p=0.033$) and torque DC ($p=0.033$) was observed. A significant increased was observed at 20-min for torque ED (+59.0±81.7%) and for torque DC (+59.0±81.7%) in the no-MVC session.

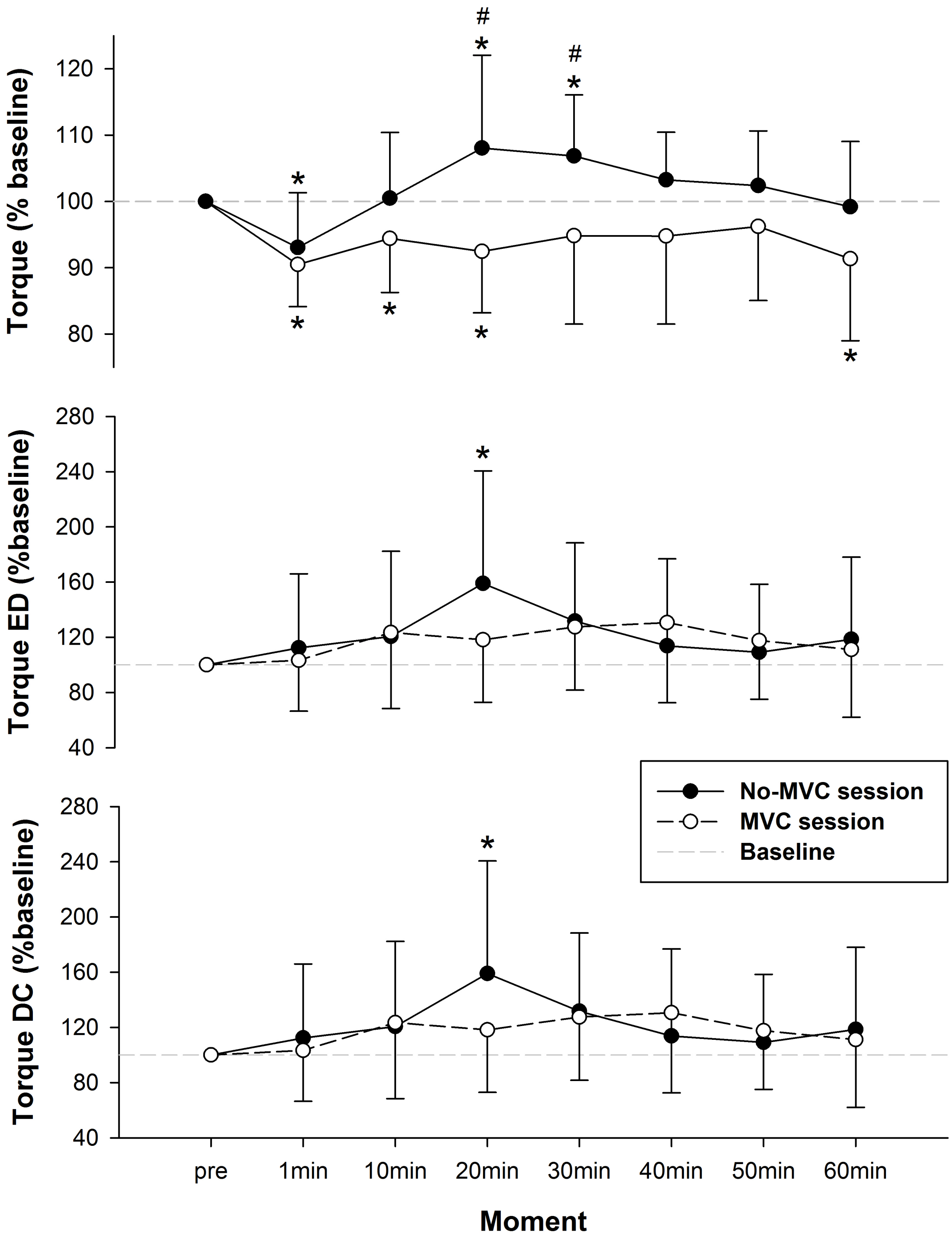

Figure 39. Torque measurements before and 1, 10, 20, 30, 40, 50, and 60 minutes after the NRI stretching protocol in both MVC and no-MVC sessions: A - ankle passive torque; B – torque hysteresis (ED); C – torque hysteresis normalized to the load stretching curve (DC).

All values are normalized to the baseline condition.

* – Statistical different from the baseline condition at $p<0.05$.

Shear wave elastic modulus. The SSI response for both stretching sessions is depicted in Figure 40. No significant effect was observed in the SSI ($p>0.464$).

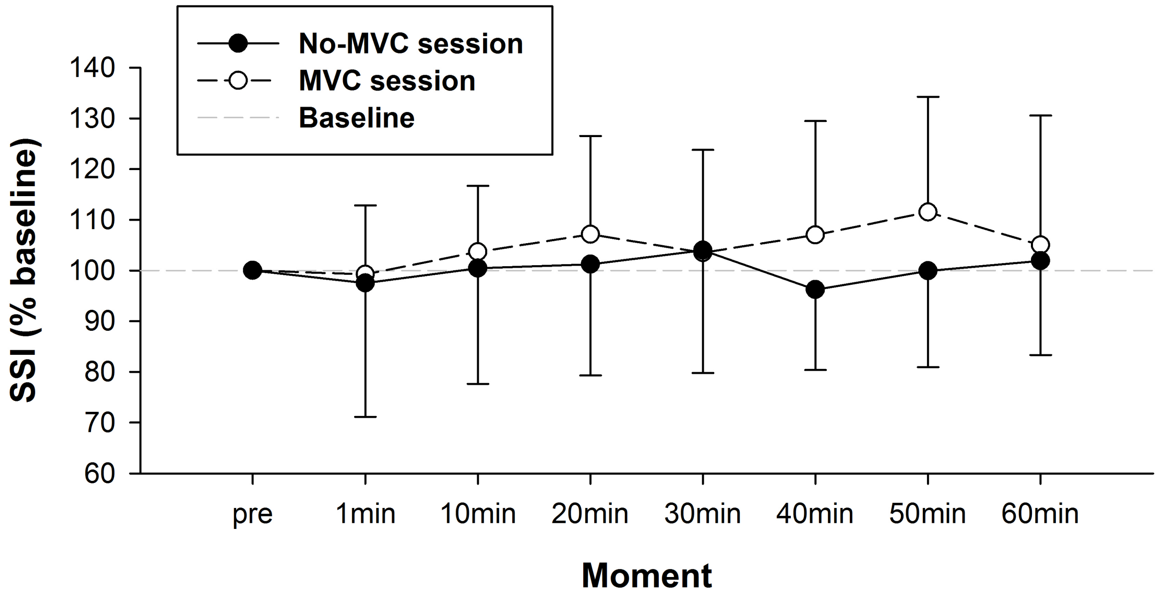

Figure 40. Shear wave elastic modulus (SSI) measurements before and 1, 10, 20, 30, 40, 50, and 60 minutes after the NRI stretching protocol in both MVC and no-MVC sessions.

Values are normalized to the baseline condition.

* – Statistical different from the baseline condition at $p<0.05$.

Maximal muscle isometric force. A significant effect was observed for time ($p=0.022$) in the MVIC for the no-MVC session only (Figure 41). No significant effect was observed for time ($p=0.48$) and protocols ($p=0.47$) in both stretching sessions. A significantly MVIC decrease was observed at 1-min (-5.0±9.3%, $p=0.04$) and 10-min (-6.7±8.7%, $p=0.02$) for the MVC session.

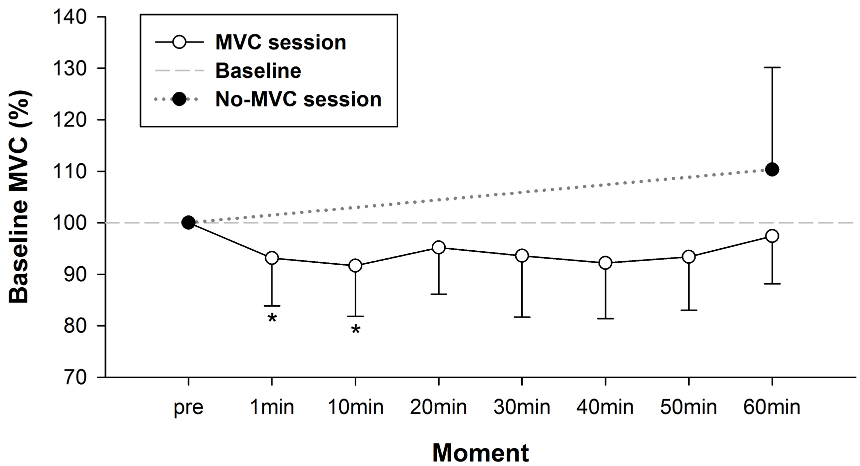

Figure 41. Ankle plantarflexors maximal voluntary isometric contraction (MVC) before and 1, 10, 20, 30, 40, 50, and 60 minutes after a NRI stretching protocol in both MVC and no MVC sessions.
Values are normalized to the baseline condition.
* – Statistical different from the baseline condition at $p<0.05$.

Discussion

This study investigated the effects of a NRI stretching protocol on ankle passive torque and muscle shear wave elastic modulus. It was observed an ankle passive torque increased at 20-min after the NRI stretching, but this was not accompanied by changes in gastrocnemius shear wave elastic modulus. This indicates that changes in passive torque are not due to changes in muscle passive tension after stretching. It was also observed an acute decrease on MVIC, but this was recovered to baseline values within one hour after stretching. This result indicates that the acute change of passive torque after the NRI stretching is not due to muscle damage.

Previous studies have often used the passive ankle torque measurements to infer about muscle passive tension and muscle stiffness (Gajdosik, 2001). However, the results of this study suggest that passive torque does not reflect changes in muscle passive tension. It was observed no changes in muscle shear wave elastic modulus, despite changes in ankle passive torque. Thus, future studies should have precaution in interpreting the meaning of passive torque changes in consequence of mechanical stimulus.

As expected as a consequence of a previous work (study 6, page 109), it was observed an increase of ankle passive torque 20-min and 30-min after a high intense stretching. In the other hand, we unexpectedly observed a passive torque decrease when isometric contractions were performed. The acute effects of muscle contractions on joint passive torque are likely to be dependent on the type of muscle contraction (Magnusson et al., 1995; Whitehead et al., 2001). However, the results of previous studies are contradictory. For instance, Magnusson et al. (1995) have reported a decrease of knee passive torque immediately after 40 knee flexors concentric contractions, and no changes were observed after 40 eccentric muscle contractions. In the other hand, Whitehead et al. (2001) reported a ankle passive torque increase 2-hours after a eccentric muscle contractions during 1-hour walking on a treadmill. Klee & Wiemann (2002) reported no significant changes in knee flexion passive torque 15-min after performing a hip flexion and knee extension resistance repetitions. Differences of the muscles studied might explain the different results.

In respect to the passive torque increase after the intense stretching, it was observed no significant changes in gastrocnemius shear wave elastic modulus. This means that the mechanism underlying the torque changes is not due to a muscle response. Thus, other tissues should be responsible for this mechanical response (e.g. connective tissue). In addition, because the torque hysteresis also increased with the increase of passive torque at 20-min and 30-min after stretching, it is reasonable to assume that this acute response also affected ankle viscosity. A previous study on the acute effect of fascia stretching (Schleip et al., 2012) observed an increase of fascia stiffness that was accompanied by a change in fascia water content. In the present study we did not measured the connective tissue passive tension during a cycle stretching, and thus we cannot confirm that hypothesis. However, it should be considered that the factor responsible for this response is suppressed by muscle isometric contractions. A previous study of Kay & Blazevich (2010) observed an Achilles tendon stiffness decrease after a bout of six concentric plantarflexors contractions. This suggests that connective tissue linked to the muscle are probably involved in the torque decrease after the NRI stretching. Future studies should examine this issue.

The second hypothesis was whether the high intensity stretching causes muscle damage, and thus increasing muscle passive tension and decreasing maximal muscle force production. Because no changes on muscle passive tension was observed, and maximal muscle force production was recovered within 20-min, we conclude that the acute response observed was not due to muscle damage. The decrease in maximal isometric force capacity has been consistently reported in the literature as a transiently and negative consequence induced by static stretching (Kay & Blazevich, 2012; Simic et al., 2013). Though, it should be noted that the average maximal plantarflexors isometric force decrease was not higher than the reported values in the literature, despite the higher intensity of the static stretching.

In conclusion, the muscle passive tension and hysteresis response to a high intense stretching do not follow the changes in joint passive torque, and intense stretching do not cause muscle damage. A practical application of this study is that joint passive torque can be reduced after a high intensity stretching when adding isometric contractions, and with maximal muscle force production restored within 20-minutes.

Study 9 – Effect of 8-week high intensity stretching training on biceps femoris long head architecture: a pilot study

Design

A randomized controlled trial was conducted to determine the effects of a knee flexor stretching intervention (Figure 7, page 43) on the biceps femoris long head (BF) architecture (Figure 19, page 83) and maximal ROM. The participants (see page 41) were allocated in two groups: a control (CG, n=5) and a high-intensity stretching training (SG, n=5). The CG were not involved in any type of stretching program during the intervention period.

Protocol

The SG performed a stretching at a ROM that corresponded to the highest tolerable torque before the onset of pain for 450 s. To assure that the maximum passive torque was obtained a NRI protocol was performed in which the ROM was increased every 90 s to a new maximal ROM. When participants reported that they could not stretch further, the knee was held statically until the end of the 450 s. We previously observed that this type of protocol achieves a greater ROM and peak torque during the stretching than a conventional rest interval protocol [see study 5 (page 101) and study 2 (page 67)]. The SG group was monitored for maximal ROM (Lafayette Gollehon Extendable, Model 01135) every training session at the beginning and during the stretching session. Experienced exercise professionals assisted the stretching maneuvers (Figure 7, page 43). Participants were asked to participate in 5 sessions per week.

Both groups were assessed for BF architecture parameters before and after the training period by an experienced researcher, using a 6-cm 10-MHz linear probe (EUB-7500; Hitachi Medical Corporation, Chiyoda-ku, Tokyo, Japan). The procedures used for image acquisition and digitizing were similar to those detailed in study 3 (page 81). A blinded researcher acquired the images, and a blinded researcher digitized the sonograms (ImageJ software, NIH, 1.47v, USA).

Statistical analysis

Data was analyzed using the SPSS software. Wilcoxon tests were used to determine pre to post effects on FL, FA, MT, and maximal ROM. Cohen's *d* coefficient was calculated to determine the magnitude of the MA and ROM changes. Statistical significance was set to $p<0.05$. Data is presented as mean±standard deviation.

Results

The SG participants performed a total of 25.0±6.4 training sessions. The average SIS score obtained during the stretching sessions for each week of training is depicted in Figure 42-A. The knee extension maximal ROM before, during, and after the stretching program are shown in Figure 42-B.

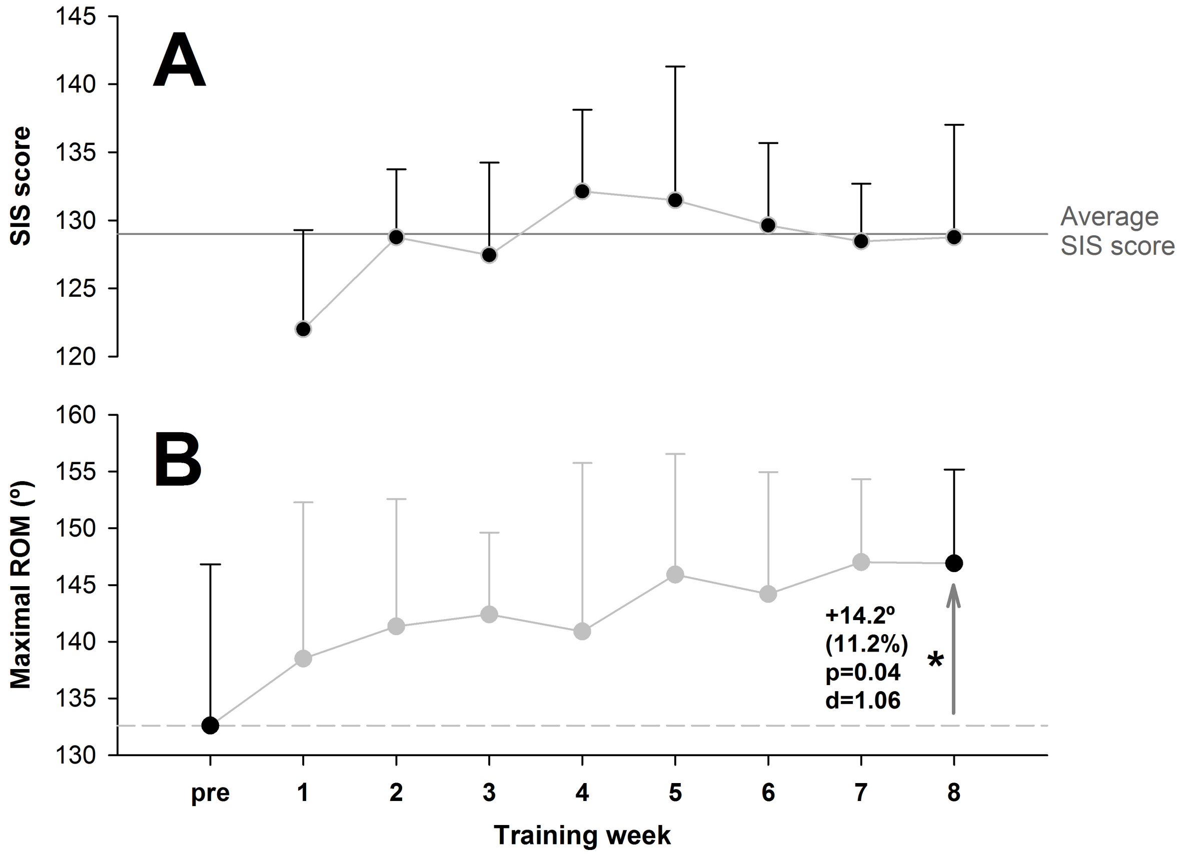

Figure 42. A) Average SIS score per week of training; B) Maximal ROM before (pre), during (gray), and after (week 8) the stretching program.

The BF architecture parameters before and after the stretching program are shown in **Figure 43**.

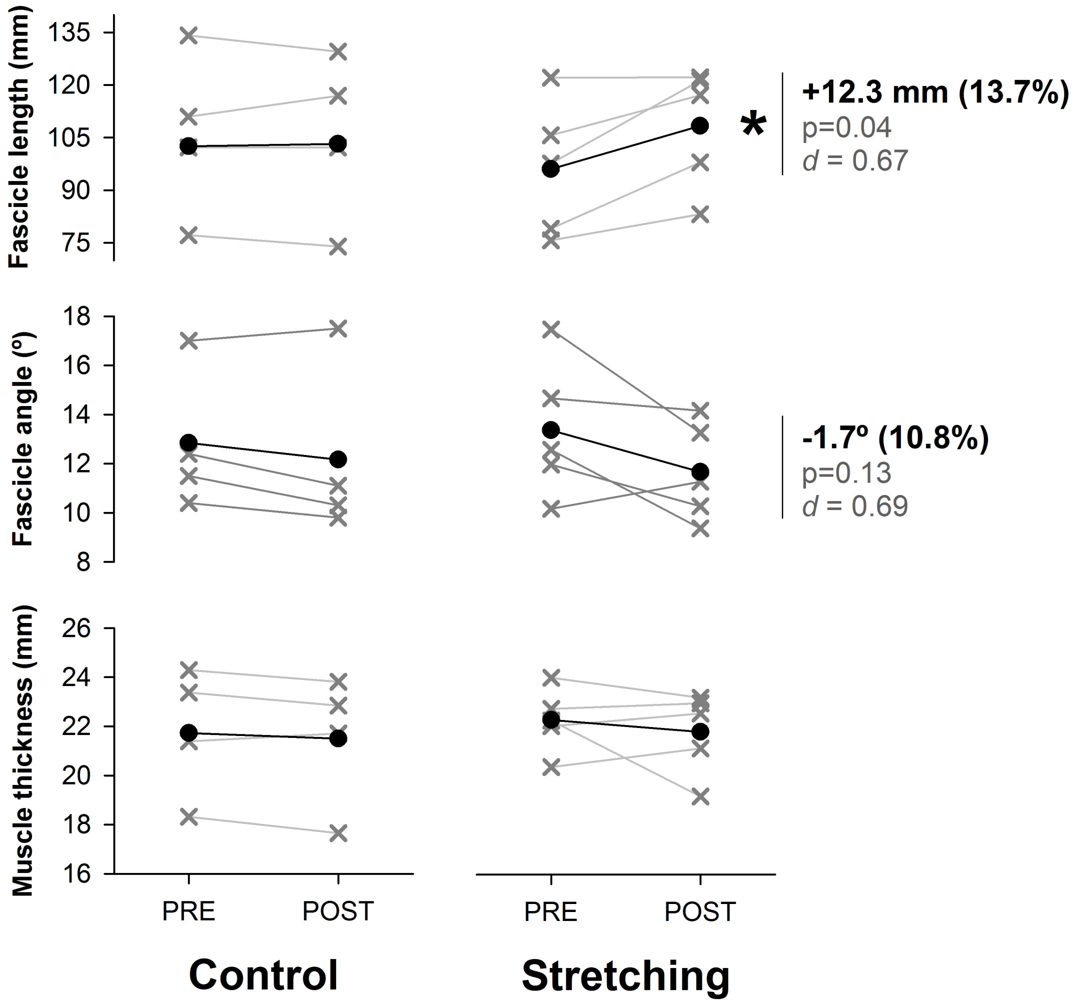

Figure 43. BF architecture parameters before (pre) and after (post) the stretching program.

Legend: *d* – Cohen's effect size coefficient; p – p-value.

* - Statistical difference at p<0.05.

Discussion

In this pilot study, the BF architecture and the knee extension maximal ROM were changed *in vivo* in consequence of an 8-week high-intensity stretching program. SG increased 13.6% of FL, and decreased 15.1% of FA (p=0.13). The FL change is meaningful, since it was an increase above the minimal detectable difference (i.e. 8.4 mm) using this ultrasound method (see study 3, page 81), and it is comparable to changes induced by resistance training (Blazevich, 2006). In respect to FA, it was expected to observe a decrease in consequence of a FL increase and no change in MT. However, the non statistical significance was observed (0.13) was due to one participant in five that did not decreased the FA, and consequently the p-value was above 0.05. This suggests a larger sample size should be used in a future study. However, it should be noted that the fascicle angle change was higher than the minimal detectable difference cut-off value using the present ultrasound assessment method (see the study 3 results section, page 86)

The previous studies examining the effects of stretching interventions have reported no significant changes in muscle architecture (Lima et al., 2014; Nakamura & Ikezoe, 2012). However, studies in animal models suggest that static stretching can change muscle architecture (Peixinho, Martins, de Oliveira, & Machado, 2014; Williams & Goldspink, 1976). We think that this might be due to the duration and intensity of the stretching intervention. Participants have stretched for 450 s in each session, and had a frequency of 3.1±0.8 sessions per week (≈1406 s of stretching for week). This duration is much higher than those used in previous studies (Lima et al., 2014; Marques et al., 2009). We also used a method that led to greater maximal ROM and torque during the stretching, and this may have been higher than in previous studies. The stretching intensity produced in the stretching protocol and measured by the SIS score was 128.6±3.0, and this is clearly higher intensity compared to a conventional rest interval stretching protocol (please see the results section of study 2, page 72).

Another observation was the ROM increases after the intervention. The total knee extension maximal ROM increase was 14.3±10.7° (+11.2±9.5%) at the end of the program. This is much higher compared to the results of previous studies examining the knee extension flexibility (Lima et al., 2014; Marques et al., 2009). We assume that this was also due to the intensity and duration of the stretching (Walter et al., 1996). In this study the ROM was increased every 90 s during the 450 s stretching maneuver until the maximal ROM, until the participant report that he could not stretch further without felling pain, and the stretching duration was superior to 300 s (Matsuo et al., 2013).

In conclusion, a high-intensity stretching program of 8 weeks was observed to efficiently increase the fascicle length and decrease the fascicle angle of the biceps femoris long head, as well to increase the knee extension maximal ROM. These findings are important to those who seek muscle architecture changes through physical training, in order to improve muscle performance. Approximately 3 stretching sessions per week, for 8-week duration, with a high stretching intensity and a duration of 450 seconds was seen to increase the fascicle length, without affecting the muscle thickness. However, since this was a pilot study, a larger sample size (i.e. >13 participants; see Study 3 on page 81) is wanted in a future study to confirm these results.

D – General discussion: link between studies

Nine studies were conducted to 1) explore and develop methodological issues for assessing the joint passive torque-angle outcomes, BF architecture, and the perception of stretching intensity (studies 1 to 3); 2) analyze the acute effects induced by stretching on joint and muscle properties (studies 4 to 8); 3) determine the long-term effects of stretching training with a high intensity on BF architecture and passive knee extension maximal ROM (study 9).

The initial issue introduced in this thesis was that previous studies examining the acute and chronic effects of static stretching using different type of protocols (e.g. different stretching durations) have assessed the joint passive torque-angle during stretching to infer about the effects on muscle passive tension-length relationship (see section 1.3 Outcomes interpretation, page 22). In addition, the torque-angle assessment has been performed during the slow passive knee extension tests in order to infer about the hamstring muscle group. The reasons for studying this muscle group has been investigated are related to injury and performance (see section 1.2 Knee extension testing protocols, page 21). However, as mention in the section 1. Joint mechanical properties (page 19), these previous studies have used different outcomes, distinctive testing conditions, and without examining which assessment condition would be the more reliable. Thus, the study 1 was conducted to determine the most reliable torque-angle outcome in the passive knee extension testing (study 1, page 53). It was mainly concluded that passive torque should be the primary outcome analyzed rather than other torque-angle parameters (e.g. the slope of the curve given point torque-angle) and should it be assessed through kinematic analysis and direct measure of the resistance to stretch. The study 3 was conducted to determine the reliability and methodological errors of the BF architecture assessment at rest using ultrasonography (i.e. fascicle length, fascicle angle, and muscle thickness), since the joint angle is a superficially mode to estimate the muscle length (page 81). This study was performed in order to use this technique in determining the long-term effects of stretching on muscle architecture. It was observed a high reliability when the same examiners acquire and digitize the BF sonograms using 6-cm image capture width. In addition, since most of previous studies examining the effects the stretching have tested for different stretching intensities, the study 2 was conducted to develop a valid and reliable instrument to assess the perception of stretching intensity in intensities bellow and above the initial maximal ROM (page 67). The results obtained in these methodological studies (i.e. 1, 2, and 3) allowed to proceed to the next studies having into account three main conclusions. First, the passive torque should be the primary end point rather of other torque-angle parameters. Second, the BF architecture could be assessed at rest with the developed ultrasound technique. And third, the perception of stretching intensity could be reliable assessed for sub- and supramaximal intensities knowing that the SIS score would be correlated to changes relative to initial maximal ROM and passive torque.

Consequently the results of study 1 were used for studies 4, 5 and 6, and the results of the study 2 and 3 were used for the study 10 (see Table 1, page 37).

The acute stretching effects on knee (studies 4, 5, and 6) and ankle (studies 7 and 8) joints properties, and on gastrocnemius muscle properties (studies 7 and 8) were also examined in this thesis. For the effects on joint passive torque, the studies allowed to analyze the effects for different intensities, different stretching intensities with different durations, and different intensities vs. durations. Consequently, intensity was analyzed from different perspectives. For muscle effects, only different intensities for equal stretching durations were compared.

The results of the present studies suggest that stretching intensity is more important for maximal ROM increase, in comparison to stretching duration. The results of studies 4, 5, and 7 support this conclusion. For instance, it was observed in study 4 that the torque decrease was similar between stretching protocols with different intensities (i.e. 100% vs. 75% vs. 50% of the tolerable torque) when compared for the same stretching duration (see Table 13, page 97). On the other hand, a higher increase on joint maximal ROM was observed for the protocol that produced the highest stretching intensity (i.e. P100). In study 5, when two stretching protocols that lead to two different stretching intensities above the initial maximal ROM (i.e. supramaximal intensities) were compared for the same time under stretching (RI vs. NRI), it was observed a higher increase on maximal ROM for the stretching protocol that produced the highest stretching intensity (i.e. NRI). In addition, and contrary to what was expected, the passive torque decrement after stretching was lower for the protocol that produced the highest stretching intensity (see Figure 27, page 106). Finally, the results of study 7 showed that the passive torque decrement was similar between two submaximal intensities (R60 and R80), and no change was observed for the lowest stretching intensity (i.e. R40). The unchanged passive torque result after the R40 protocol stretching was contrary to what we initially expected, because the results of study 4 showed equal changes for between the intensities P50 and P75. We do suggest two hypotheses to explain these differences: 1) the joint passive torque responses to stretching may be different between the ankle and the knee; 2) the effects of intensity on joint torque are similar when these stretching intensities are performed above a certain threshold stretching intensity. The first hypothesis is based on the results of previous studies that showed different joint responses to stretching between the ankle and the knee (Fowles et al., 2000; Herda et al., 2012). The second hypothesis is suggested because the intensity of R40 in the study 7 was less than 38% of maximal tolerable dorsiflexion passive torque, and this value is much lower than the lowest stretching intensity performed in the study 4 (i.e. 50% of tolerable knee extension passive torque). Thus, it is possible that the stretching effects are only attained when a certain degree of stretching intensity is performed.

The study 4, also allowed us to infer about the effect of duration in different stretching intensities. For instance, it was observed that the passive torque decrease was higher with longer durations and equal torque decrease was obtained when similar stretching durations were performed across the protocols (i.e. 87.6% after 180 s of stretching for P50, 89.0% after 135 s of stretching for P75, and 85.8% after 180 s of stretching for P100). These results suggest that the stretching duration extents the torque decrease in any stretching intensity. In respect to the effects on maximal ROM, none of the previous studies allow to conclude about the effects of duration in different stretching intensities.

Regarding the effects of combined different intensities and durations, two protocols with an inverse proportion of intensity and duration were compared in the study 6 (page 109): high-intensity and short-duration (HISD) vs. low-intensity and long-duration (LILD). It was observed that intensity was a key training variable to acutely increase the maximal ROM and peak torque, and duration was more important for acute passive torque decrease. This conclusion is taken since no increase in maximal ROM was observed for LILD but a higher immediate passive torque decrease was obtained compared to HISD for at least 60 minutes after stretching. The same conclusion for effects on passive torque is supported by the results of the study 4 (page 91). It was observed that the lowest intensity and longer duration (P50 = 50% of tolerable passive torque and 180 s) had a higher passive torque decrease (-22.2%) compared to the protocol with higher intensity and shorter duration (-13.4%; P100 = 100% of tolerable passive torque and 90 s) at the end of one stretching repetition.

Moreover, in study 6 the observations for the effects on joint passive torque and maximal ROM were performed at 1, 30, and 60 minutes after the stretching. An unexpected effect observed after the HISD stretching was the passive torque increase above the baseline. Such effect did not occurred for a lower stretching intensity and long duration. In order to confirm this result and to extent for the mechanisms underlying this response, the study 8 was conducted targeting the ankle plantarflexors with a high intensity and short duration stretching using a NRI protocol. It was also observed an increase of joint torque at 20 minutes after stretching that was accompanied with an increase of the torque dissipation coefficient (Figure 39, page 132), and thus confirming the passive torque increase above baseline after the stretching can be observed when a high intensity is performed using a NRI protocol. In addition, it was seen in study 8 three more observations: 1) no significant changes in muscle shear elastic modulus of one of the muscles stretched (i.e. gastrocnemius) occurred; 2) the plantarflexors MVIC was recovered within one hour; and 3) the passive torque increase was suppressed when isometric contractions were performed between post stretching assessments (i.e. every 10 minutes). These results suggest that the joint passive torque response is not attributable to the muscle component but to the connective tissue surrounding and linked to the muscle. We conclude this since no changes have occurred in shear elastic modulus (i.e.

muscle passive tension), no muscle damage have occurred after the NRI protocol (i.e. the MVIC was recovered within 60 minutes), and the passive torque increase was suppressed by the post stretching isometric contractions. In addition, it should be considered that connective tissues it is known to mediate the force transmission from the muscle to the bones or between muscles during the muscle contraction and passive stretching (Bojsen-Møller et al., 2010; Huijing & Jaspers, 2005; Yucesoy, Maas, Koopman, Grootenboer, & Huijing, 2006).

Regarding the muscle acute responses, two studies (7 and 8) were conducted to analyze the effects induced by different stretching intensities. In study 7, observations were performed during (i.e. stress relaxation) and immediately after the stretching for the ankle plantarflexors for three intensities. During the SR, a higher absolute and relative shear elastic modulus decrease was seen for the greatest stretching intensity (R80), with no changes in fascicle length for all stretching intensities.

Immediately after the stretching no significant changes were observed for shear elastic modulus, except for the protocol R80 at the angle corresponded to 75% of ROM (i.e. approximately 32.8º of dorsiflexion), whereas a significant decrease was found. This result suggested that the changes in muscle shear elastic modulus were only attained for higher stretching intensities and did not reflect in all `shear elastic modulus-ROM´ curve, only in the final portion of the curve. However, when we stretched for a higher intensity using a NRI protocol (study 8), no significant changes in shear elastic modulus were observed within one hour after stretching. We think that no changes were detected in the shear elastic modulus after the NRI protocol because effects were observed for a small dorsiflexion ROM (i.e. less than 20º). If we assume that the shear elastic modulus changes only occur at longer muscle lengths, then it was not possible to conclude whether the shear elastic modulus alters as a result of stretching with greater intensity because the ROM tested was small. As such, this matter should be further examined in future studies.

Another finding observed in studies 7 and 8 was related to the mechanical effects observed in the joint and the muscle. As previously mentioned (see section 1. Joint mechanical properties, page 19), the passive torque-angle has been used to infer about the effects on the muscle passive force-length relationship after stretching interventions. However, the results of studies 7 and 8 suggest that the torque responses do not reflect changes in muscle passive tension and stiffness. For instance, in study 7 it was observed that the passive torque SR normalized to peak torque (i.e. torque at the beginning of SR) was similar across intensities, but on the other hand the relative muscle shear elastic modulus SR was higher for greater intensities and did not occur for the lowest stretching intensity (see **Figure 33**, page 120). In addition, the stretching interventions in study 7 induced a decrease in relative passive torque for some intensities and in some ankle angles that did not change the muscle shear elastic modulus. A similar observation was found in study 8, whereas the NRI acutely decreased the joint passive torque without significant changes in muscle shear elastic modulus. Consequently, because the torque-angle measurement represents a net force of several structural factors comprising the joint being deformed, the mechanical effects on joint and muscle should be assessed throughout different methodologies.

In consequence of the studies 4, 5, and 6 results it was decided to examine the long-term effects of stretching in study 9 using the highest stretching through the use of a NRI protocol. The NRI was chosen based on the results obtained in the study 5 (page 101), where it was observed that the NRI produced a greater stretching intensity across and after stretching repetitions compared to a RI stretching protocol. It was assumed in study 9 that a greater acute increase on maximal ROM would theoretically induce a greater chronic maximal ROM gain. Consequently, it was observed in the pilot study 9 an average increase 14.3° (+11.2%) after an 8-week intervention, and this value was higher compared to the results of previous studies (please see study 9 discussion, page 139). This reinforces the initial premise that intensity is a key variable for increasing the joint maximal ROM.

Another issue explored in study 9, was the changes in muscle architecture in consequence of high intensity stretching training. This study was accomplished because it has been theoretically assumed that if passive torque changes at a given joint angle after a stretching intervention, this would reflect changes in the muscle passive tension at a given muscle length, due to an increase of muscle length (please see section 4.2 Chronic on page 34; please see Figure 5 on page 35). Since the passive torque does not reflect muscle passive tension or either angle reflects the muscle length, it was decided to measure the muscle fascicles length. The study 9 was proceed by the methodological study 3 (page 81) in which developed a reliable technique to assess the BF architecture using ultrasonography. It was seen in study 3 that reliability was high when assessment was performed with the same examiners acquiring and digitizing the sonograms using a 6-cm sonogram width. In addition, it was determined the minimal detectable difference (MDD) for the different architectural parameters that should be considered as the cut-off to determine a real adaptation in consequence of an intervention training. The 8-week stretching training significantly increased the fascicle length (+12.3 mm, 13.7%, $p=0.04$) and non-significantly decreased the fascicle angle (-1.7º, 10.8%, $p=0.13$); with both outcomes having values above the MDD values (FL = 8.4 mm; FA = 1.5°). As mentioned in the discussion of study 9, we think that the changes of biceps femoris FL and FA are consequence of a higher stretching intensity. Because we previously hypothesized that the degree of stretching intensity could determine the changes on muscle architecture, it necessary to have an instrument to assess the stretching intensity. As such, before the study 9 it was conducted a study (2) to develop a valid and reliable scale to assess the perception of stretching intensity (page 67). In this way, it was possible to know how much intense was the stretching maneuvers during the intervention, having the initial maximal ROM within a session as a reference. The previous studies examining the effects of stretching on muscle architecture have used protocols with rest intervals between repetitions, and a duration lower to those used in study 9. As can be seen in the results section of the study 2 (page 72), the SIS score is higher for the NRI protocol compared to the RI condition, and this accompanied with a greater ROM and peak torque was obtained in the NRI. In the study 9, the average SIS score observed using the NRI protocol was 128.6±3.0, and this is greater than the average value found for RI protocol in study 2 (119.1±19.0).

The fascicle length increase implies that the number of sarcomeres in series also rises. The studies on animal models have previously demonstrated that the number of sarcomeres in series increases after a lengthening stimulus (Peixinho et al., 2014; Williams & Goldspink, 1976), and consequently this is thought to increase the fascicle length. In addition, previous observations suggest that the change in the number of sarcomeres increase depending on the degree of the lengthening stimulus (Lindsey et al., 2002). This insinuates that fascicle length increase in consequence of a training stimulus in stretching intensity dependent. The results of study 9 support this premise.

A novel aspect of this thesis was the scale developed to assess the perception of stretching intensity (study 2, page 67). It was observed that the SIS score was correlated to changes in passive torque and joint ROM. Since it was seen in the studies of the present thesis that different intensities produce different effects on joint and muscle properties, a scale to assess the perception of stretching intensity becomes useful from a practical point of view, as such in physical training or clinical settings. In addition, an original aspect of this scale is the fact of having a supramaximal intensity component (see Figure 16, page 76), having the maximal ROM within a training session as a reference for the maximum. Since it has been demonstrated that the ROM can be acutely increased above its initial maximum across a number of stretching repetitions and through the use of certain methodological procedures (see study 5, page 101), the scale becomes useful in detecting these changes. Also, the scale reveals to be useful through the discrimination of the intensity changes when using methodological procedures that lead to distinct intensities of ROM and peak torque, as using or not rest intervals between repetitions (see Figure 17, page 77).

Summarizing, the study 1 allowed to conclude that passive torque outcome should be used in studies 4, 5, 6, 7, and 8 to analyze acute joint mechanical responses in a systematic stretching protocol; from studies 4 to 8 it was observed that both joint and muscle responses depend on stretching intensity, as well as chronic changes on muscle architecture are induced by 8-weeks of stretching with high intensity (study 9), using a reliable ultrasound muscle architecture assessment (study 3), and assuring that stretching intensity was higher compared to conventional static stretching protocols (study 2).

E –Limitations and suggestions

The present thesis should be framed in some studies limitations. Hereunder are presented the limitations identified in the studies conducted for this thesis, as well as the justifications and suggestions for future studies.

A general studies consideration was the way that the stretching intensity was determined. For instance, in studies 4 and 6 the submaximal intensity was determined as a percentage of maximal tolerable torque, in studies 5 and 8 the supramaximal intensity was determined according to the stretch tolerance to highest ROM, and in study 7 as a percentage of maximal ROM. Consequently, this different procedure made it difficult to perform some comparisons between studies (i.e. compare the immediate stretching effects on joint passive torque between the P50 of study 4 vs R40 of study 7).

Another consideration was that intensity was not the only dependent variable that was tested in some studies (i.e. studies 4 and 6), but was tested together with the stretching duration. The studies were designed in this manner so stretching intensity could be analyzed by it self, with different stretching durations, and using different proportions of \`intensity and duration´.

In study 2, the stretching intensity scale was developed and tested for validity and reliability in order to assess the perception of stretching intensity. However, this scale was only tested for male participants. This was done because it was known that the perceived exertion responses during physical activities are different between genders. In addition, the SIS was validated only for one stretching maneuver and involving the knee joint. Thus, in order to give a global property to use the SIS, future studies should validate the scale with other populations and testing in other joints.

In study 3, the BF architecture was only assessed in the muscle mid-belly and in a rest muscle condition in which the knee was full extended and the hip was in a neutral position. This means that the assessment is specific to the portion of the muscle being studied and cannot be used to represent the overall BF muscle architecture, since there is evidence that BF architecture is heterogeneous along the muscle (see section 2.1 Architecture, page 23). Thus, future studies should develop different techniques to assess BF architecture in other different portions.

Regarding the study 4, it must be considered that the different protocols had different number of rest intervals. This was performed in this way so it could be possible to simultaneously compare among stretching intensities at different durations (i.e. by performing the five repetitions) and for different durations (i.e. for the number of repetitions that totalized the same time under stretch across protocols). Thus, future studies may want to have a higher strict control over these variables.

In respect to the study 5, two aspects should be considered: first, the number of stretching repetitions was different among the participants; second, few studies performed 4 and 5 repetitions in the NRI condition. Regarding the first consideration, the number of NRI repetitions has varied because the stretching tolerance was different among participants. Since the criterion to increase ROM was to perform the stretching without pain, the participants responded different in respect to the number of NRI repetitions. However, the conclusions of the study were not affected since the comparisons between protocols were performed for the same number of repetitions. Regarding the second aspect, it was impossible to predict how many repetitions would produce each participant, and few participants tolerated more than 3 NRI repetitions. Consequently, in order to have a significant number of subjects performing 4 or more NRI repetitions, the sample size should increase in further studies.

As mentioned before, the determination of the stretching intensity was not consistent among studies. In respect to study 6, the intensity was based on the tolerated torque. However, because in the NRI protocol (i.e. HISD condition) both the ROM and passive torque increases every 90 s after obtaining the maximal ROM, the intensity among performed was not proportional. The same consideration should be done to the duration, since the number of NRI repetitions was different across participants. It was decided to use a NRI protocol because it was previously seen that this protocol produces a higher intensity compared to a conventional rest interval protocol and the aim of the study was to compare the highest supramaximal intensity to a moderate submaximal intensity. Despite these limitations, the study objective was not affected.

Finally, the study 9 was initially conceived based on the evidence that the long-term effects of stretching would reflect a decrease of the joint passive torque (see section 4.2 Chronic, page 34), and this indirectly suggests that the muscle adapts by increasing its length and consequently originates less passive tension at a given joint angle (see Figure 5, page 35). However, the previous studies reporting a decrease of passive torque have assessed this variable when the joint was under load (i.e. stretching), and not in a resting condition. The assessment of BF fascicles length was performed in a resting state and not at a given joint angle under load. However, it has been assumed that changes in fascicle length would be detected in both rest and lengthening conditions (Blazevich, 2006). Thus, the findings of study 9 were not biased.

As a consequence of the results obtained in the studies of this thesis, three main issues are proposed to be examined in future studies. The first is related to the acute and chronic mechanical effects of different stretching durations at different stretching supramaximal intensities. Since the intensity performed above the initial maximal ROM was seen as a key variable for increasing flexibility and changing muscle architecture, it would be important to know to what extent the duration at these stretching intensities potentiates these mechanical adaptations. The second suggestion is related to the criterion to determine the maximal ROM, and to relate this to physiological variables. As mentioned in section 3.2 Maximal range of motion (page 27), various criteria has been used in previous studies to assess the maximal ROM. However, it is unknown which criteria would best represent the participant maximal ROM in a reliable manner. Such information would be useful for clinical and research settings, as help to improve some methodological procedures such as the assessment of stretching intensity using the SIS.

F – Conclusions

The main conclusions of this thesis can be separated into three issues: methodological, acute effects, chronic effects. It was concluded that:

Methodological

1) Passive knee extension torque-angle measurements should be performed using 2D kinematic analysis coupled and directly measuring resistance to stretch directly rather than isokinetic dynamometer for higher assessment reliability.

2) Precautions with and strict control of the position of non-tested body segments should be taken by researchers because they can affect torque-angle outputs.

3) Test-retest reliability is clearly higher for the passive torque outcome compared to the slope of the torque-angle curve or the parameters of the fitted model to torque-angle raw data.

4) A new, reliable, and valid scale was developed to assess the perception of stretching intensity, and this is thought to be useful for clinical interventions or research settings.

5) A single, still-image ultrasonography can be used for the reliable assessment of BF architecture in the muscle mid-belly with high reliability when the image acquisition and digitizing is performed by the same examiner and the 6 cm imaging window width is used.

Acute effects

6) A higher stretching intensity potentiates the acute joint range of motion gains, and the stretch duration potentiates the acute passive torque decrement.

7) Non-resting between stretching repetitions increases the efficiency in increasing the joint maximal range of motion and tolerable passive torque, but in the other hand do not potentiates the decrease in passive torque at a given angle.

8) A high intensity stretching through the use of a non-rest interval stretching protocol increases the joint passive torque at a given angle 30 minutes after the stretching.

9) The passive torque response 30 minutes after a intense stretching seems to be related to connective tissue and not a skeletal muscle response.

10) The muscle stress relaxation and the acute response to static stretching seems to be dependent on the stretching intensity.

11) Muscle architecture is unaffected during and after the static stretching at different submaximal intensities.

Chronic effects

12) A stretching intervention for 8-weeks with a high intensity was seen to increase both the maximal passive knee extension of range of motion and the biceps femoris long head fascicles; however, since this

observation was obtained in a pilot study with a small sample size, further studies may want to confirm this result with a larger number of participants.

H – References

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I – Annexes

1. Instruction for SIS administration

1.1 Before the stretching

A presente escala serve para quantificar a intensidade com que sente o alongamento. Durante este exercício **[indicar aqui o nome/descrição do exercício]**, deverá sentir alongamento aqui **[apontar o local do corpo onde deve sentir o alongamento]**. Ao alongar deverá sentir maior tensão nestes tecidos.

A escala apresenta duas componentes: uma submaximal com pontuação de 0 a 100 **[apontar para a escala]**, e outra supramaximal para pontuações acima de 100 **[apontar para a escala]**. O valor 100 significa a intensidade de máximo alongamento sem sentir dor,[13] e o valor 0 significa sentir nenhum alongamento. Ao longo da escala estão apresentados descritores/palavras junto a expressões numéricas, que caracterizam a intensidade do alongamento. Exemplo: **[descrever aqui um exemplo de uma palavra associada a um número e a uma posição na componente visual da escala]**

Deverá indicar a intensidade com que sente o alongamento sempre de acordo com os valores da escala, quer para intensidades abaixo do seu máximo, quer para intensidades acima do máximo, considerando o máximo o valor da primeira repetição.[14]

Quando lhe apresentar a escala na sessão de alongamento, deverá primeiro olhar para a escala, escolher um **termo** que melhor caracteriza a intensidade com que sente o alongamento, e só depois indicar um **número** que caracterize a intensidade que sente o alongamento. Exemplo: **[descrever aqui um exemplo]**[15]

É importante que responda com a máxima sinceridade e precisão, em função da intensidade de alongamento que sente.

Alguma dúvida ou questão?

1.2 During the stretching

Olhando para a escala, indique primeiro o termo e depois o número que descreve a intensidade que sente no alongamento.

1.3 For producing supramaximal intensities

Produza o maior grau de alongamento sem sentir dor ou desconforto.

[13] In the studies 2 and 10 it was used this reference to define the maximal range of motion. Nevertheless, other references may be used.
[14] It is important to have a reference for the maximal ROM of the participant. Thus, we recommend that this should be performed first.
[15] A simple and short example should be given to the participant (e.g. moving the finger to the submaximal and maximal ROM).