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# Biomechanical properties of skeletal muscle and peripheral nerve

## Tissue and joint adaptations to acute and chronic stretch interventions

## JURY

| | |
|---|---|
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## LIST OF ABBREVIATIONS

| | |
|---|---|
| EMG | Electromyography |
| e.g., | *Exempli gracia* (for example) |
| i.e., | *id est* |
| MTU | Muscle-tendon unit |
| RMS | Root mean square |
| ROM | Range of motion |
| GL | *Gastrocnemius lateralis* |
| GM | *Gastrocnemius medialis* |
| SOL | *Soleus* |
| SEM | Standard error of measurement |
| SSI | Supersonic shear imaging |
| TBL | Tibial nerve |

# PUBLICATIONS AND CONFERENCE PRESENTATIONS

### *Published peer-reviewed articles related to this thesis*

**Andrade RJ**, Lacourpaille L, Freitas SR, McNair PJ & Nordez A. (2016). Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension. *Scandinavian journal of medicine & science in sports* 26, 41-47.

**Andrade RJ**, Nordez A, Hug F, Ates F, Coppieters MW, Pezarat-Correia P & Freitas SR. (2016). Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography. *Journal of biomechanics* 49, 326-331.

### *Articles currently submitted or in preparation related to this thesis*

**Andrade RJ**, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Gross R, McNair PJ & Nordez A. Sciatic nerve stiffness plays a key role in the limitation of maximal ankle dorsiflexion range of motion. *(Submitted). Medicine & Science in Sports & Exercise*.

**Andrade RJ**, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Quillard JB, Gross R, & Nordez A. Localized muscle and nerve stiffness adaptations following a 12-weeks of specific stretch training. *(In preparation).*

## *Conference presentations related to this thesis*

**Andrade RJ**, Freitas, SR, Hug F, Le Sant G, Lacourpaille L, Gross R, McNair P, Nordez A. (in preparation). Sciatic nerve stiffness influences the maximal ankle range of motion. *XXVI Congress of the International Society of Biomechanics*. July 2017, Brisbane, Australia.

**Andrade RJ**, Freitas S, Ates F, Hug F, Nordez A. Non-invasive evaluation of sciatic nerve stiffness during ankle motion in humans. *16th ACAPS International Congress*. October 2015 Nantes, France.

***Other published peer-reviewed articles***

Nordez A, Gross R, **Andrade RJ**, Le Sant G, Freitas SR, Ellis R, McNair PJ & Hug F. (2017). Non-Muscular Structures Can Limit the Maximal Joint Range of Motion during Stretching. *Sports Med* [Epub ahead of print].

Freitas SR, Mendes B, Le Sant G, **Andrade RJ**, Nordez A & Milanovic Z. (2017). Can chronic stretching change the muscle-tendon mechanical properties? A review. *Scandinavian journal of medicine & science in sports* [Epub ahead of print].

Neto T, Freitas S, **Andrade RJ**, Gomes J, Vaz J, Mendes B, Firmino T, Nordez A & Oliveira R (2017, accepted). Sciatic nerve stiffness is not changed immediately after a slump neurodynamics technique. *Muscles, Ligaments and Tendons Journal*.

Dieterich AV, **Andrade RJ**, Le Sant G, Falla D, Petzke F, Hug F & Nordez A. (2017). Shear wave elastography reveals different degrees of passive and active stiffness of the neck extensor muscles. *European journal of applied physiology* 117, 171-178.

Lacourpaille L, Nordez A, Hug F, Doguet V, **Andrade RJ** & Guilhem G. (2017). Early detection of exercise-induced muscle damage using elastography. *European journal of applied physiology* [Epub ahead of print].

Neto T, Freitas SR, Marques M, Gomes L, **Andrade RJ** & Oliveira R. (2017). Effects of lower body quadrant neural mobilization in healthy and low back pain populations: A systematic review and meta-analysis. *Musculoskeletal Science and Practice* 27, 14-22.

Le Sant G, Nordez A, **Andrade RJ**, Hug F, Freitas S & Gross R. (2017). Stiffness mapping of lower leg muscles during passive dorsiflexion. *Journal of anatomy* 230, 639-650.

Helfenstein-Didier C, **Andrade RJ**, Brum J, Hug F, Tanter M, Nordez A & Gennisson JL. (2016). In vivo quantification of the shear modulus of the human Achilles tendon during passive loading using shear wave dispersion analysis. *Physics in medicine and biology* 61, 2485-2496.

Freitas SR, Vaz JR, Bruno PM, **Andrade RJ** & Mil-Homens P. (2016b). Stretching Effects: High-intensity & Moderate-duration vs. Low-intensity & Long-duration. *International journal of sports medicine* 37, 239-244.

Freitas SR, **Andrade RJ**, Nordez A, Mendes B & Mil-Homens P. (2016). Acute muscle and joint mechanical responses following a high-intensity stretching protocol. *European journal of applied physiology* 116, 1519-1526.

Freitas SR, Vaz JR, Bruno PM, Valamatos MJ, **Andrade RJ** & Mil-Homens P. (2015). Are rest intervals between stretching repetitions effective to acutely increase range of motion? *International journal of sports physiology and performance* 10, 191-197.

Freitas SR, **Andrade RJ**, Larcoupaille L, Mil-homens P & Nordez A. (2015). Muscle and joint responses during and after static stretching performed at different intensities. *European journal of applied physiology* 115, 1263-1272.

**Andrade RJ**, Freitas SR, Vaz JR, Bruno PM & Pezarat-Correia P. (2015). Provocative mechanical tests of the peripheral nervous system affect the joint torque-angle during passive knee motion. *Scandinavian journal of medicine & science in sports* 25, 338-345.

Silva A, Manso A, **Andrade RJ**, Domingues V, Brandao MP & Silva AG. (2014). Quantitative in vivo longitudinal nerve excursion and strain in response to joint movement: A systematic literature review. *Clinical biomechanics* 29, 839-847.

## *Articles currently submitted and under peer-review*

Le Sant G, Nordez A, Hug F, **Andrade RJ**, Lechart T, McNair P, Gross R. Lower leg muscle stiffness in stroke survivors during passive dorsiflexion. *(Submitted). Arch Phys Med Rehabil.*

Ates F*, **Andrade RJ***, Freitas S, Hug F, Lacourpaille L, Gross R, Yucesoy C, Nordez A. Mechanical interactions between human lower leg muscles in vivo shown using elastography. *(In revision). European journal of applied physiology*.

Neto T, Freitas S, **Andrade RJ**, Vaz JR, Mendes B, Firmino F, Bruno PM, Nordez A, Oliveira R. Sciatic nerve stiffness is increased in people with low back related leg pain and can be acutely reduced using a neural tension technique. *(In revision). J Bodyw Mov Ther*.

** These authors contributed equally to this work.*

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# ABSTRACT

Maximal joint range of motion (ROM) of a joint, or joint flexibility, is an important functional outcome often used in clinical practice, sports and research designs to estimate the maximal length of passive muscle-tendon unit (MTU). Maximal ROM was thought to be mainly restricted by the tension developed by MTUs being stretched during articular motion (mechanical-based theory). However, there is a growing body of experimental research suggesting that stretch training induces increases in maximal ROM in the absence of significant mechanical adaptations in MTU. Therefore, sensory-based theory of muscle extensibility has emerged to explain the mechanisms underlying the changes in maximal ROM. More recently, non-muscular structures such as peripheral nerves and fasciae have been pointed to limit the stretching amplitude and thus the magnitude of passive muscle tension that can be applied during stretching maneuvers. However, direct evidence is lacking. This doctoral research aimed to extend the knowledge about the mechanical role of muscular and non-muscular tissues underpinning the limitation of maximal ROM *in-vivo*. In particular, we hypothesized that, among the non-muscular structures, peripheral nerves can play a key role in joint flexibility. Taking advantage of ultrasound shear wave elastography to assess the passive stiffness of individual muscles and nerve, four studies were conducted to: 1) examine whether mechanical loading of muscular and non-muscular structures limit the maximal ROM; and 2) if the mechanical properties of muscles and peripheral nerves adapt to both acute or chronic stretch training. The findings from this thesis show that maximal ROM can be limited by either muscular or non-muscular structures, depending on the posture of the entire lower limb which is decisive to stretch preferentially the target tissue. Acute and chronic stretching targeting separately each structure were shown to induce changes in mechanical properties of muscles and peripheral nerves. In addition, improvements in maximal ROM were specific to the tissues that were more loaded and correlated with the changes in their mechanical properties.


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***Key-words:*** *range of motion; stiffness; ultrasound shear wave elastography; peripheral nerves; muscle; ankle; passive torque*

# RESUME

L'amplitude articulaire maximale (AAM) peut être considérée comme faisant partie intégrante du mouvement humain. L'AAM influence les activités fonctionnelles et les performances sportives, peut avoir un impact sur le risque de blessures et évolue notamment avec le vieillissement ou l'immobilisation (Akeson *et al.*, 1987; Trudel *et al.*, 1999; Mulholland & Wyss, 2001; Witvrouw *et al.*, 2003; Hemmerich *et al.*, 2006). Alors que l'AAM active est générée par l'action de l'unité muscle-tendon agissant comme un levier sur l'articulation, l'AAM passive est la quantité de mouvement que peut parcourir l'articulation en étant déplacée en l'absence d'activité musculaire significative du sujet (i.e. par un tiers) (Gajdosik, 2001).

Traditionnellement, lorsqu'on évalue la mobilité articulaire en conditions passives, la limitation de l'AAM est attribuée à deux entités principales : les articulations et les muscles. Pour certains mouvements (e.g. extension du genou, extension du coude), il est évident que la congruence articulaire et les structures intra-articulaires telles que la capsule sont les principales structures limitant l'AAM. Pour d'autres articulations (e.g. hanche, cheville), l'AAM est principalement limitée par la résistance développée par les unités muscle-tendon étirées en réponse au mouvement articulaire (Herbert, 1988; Magnusson, 1998; Gajdosik, 2001; Weppler & Magnusson, 2010). Cette réponse passive dépend à la fois des propriétés structurelles et mécaniques du muscle-tendon ainsi que des enveloppes conjonctives dont les propriétés viscoélastiques remarquables fournissent la plupart de la tension passive. Cependant, plusieurs études suggèrent que, au niveau du membre inférieur, l'AAM de la cheville puisse être impactée selon certaines configurations posturales du membre inferieur, sans pour autant déclencher des adaptations mécaniques et structurelles au sein de l'unité muscle-tendon étirée (Weppler & Magnusson, 2010; Freitas *et al.*, 2017; Nordez *et al.*, 2017).

En effet, pendant la course articulaire, certains tissus non-musculaires tels que les nerfs périphériques et les fascias peuvent également subir de grandes modifications de longueur et développer une réponse résistive au mouvement. Cependant, l'influence de ces tissus non-musculaires sur l'AAM reste largement inexplorée. Par exemple, des diminutions significatives de l'AAM de la cheville ont été observées lors du changement de positionnement d'autres articulations (e.g. hanche) et pourtant non liées par des structures musculo-squelettiques (Gajdosik

*et al.*, 1985; Mitchell *et al.*, 2008; Palmer *et al.*, 2015). De plus, il a récemment été montré que les fascias et les nerfs périphériques, qui sont richement innervés, peuvent subir des changements dans leur longueur pendant le mouvement articulaire (Silva *et al.*, 2014; Cruz-Montecinos *et al.*, 2015). Ainsi, l'AAM peut être limitée dans certaines postures corporelles qui étirent des structures non-musculaires. Cependant, il existe actuellement un manque de preuves scientifiques pour quantifier, de façon plus directe, dans quelle mesure les propriétés mécaniques des structures non-musculaires peuvent affecter l'AAM.

Plus précisément, il a été observé pendant la réalisation de certains tests cliniques mobilisant les nerfs périphériques que l'AAM est considérablement limitée (Gajdosik *et al.*, 1985; Laessoe & Voigt, 2004; McHugh *et al.*, 2012; Andrade *et al.*, 2015). Des études cadavériques ont montré une augmentation de la raideur dans le nerf tibial au niveau du tunnel tarsien, sans affecter d'autres structures musculo-squelettiques liées à la cheville (Coppieters et al., 2006). De plus, aucune étude n'a évalué de façon non-invasive les propriétés mécaniques des nerfs périphériques *in-vivo* en réponse au mouvement articulaire.

Les évaluations classiques par la mesure du couple passif selon l'angle articulaire sont classiquement utilisées pour étudier *in-vivo* les mécanismes mécaniques sous-jacents à l'AAM. Cependant, ces tests ne sont pas suffisamment sensibles pour détecter de petits changements mécaniques dans les tissus musculaires et non-musculaires. De plus, il a été suggéré que les tissus non-musculaires doivent avoir une faible contribution sur couple passif (Nordez et al., 2017). Spécifiquement, les relations couple passif-angle articulaire ne fournissent ni des informations sur la géométrie musculaire (i.e., modifications de longueur), ni une quantification directe de la raideur tissulaire individuelle durant l'étirement. Pourtant, ces indications sont essentielles pour une compréhension exhaustive des propriétés mécaniques des structures musculaires et non-musculaires qui sous-tendent la limitation de l'AAM, notamment après une intervention (e.g. étirement).

Le développement de techniques d'imagerie telles que l'échographie a permis d'évaluer les architecture de l'unité muscle-tendon chez l'homme, *in-vivo* et de façon non-invasive. Plus récemment, l'échographie classique et l'élastographie par ondes de cisaillement ont permis d'évaluer la raideur des tissus biologiques avec une bonne fiabilité (Bercoff et al., 2004; Gennisson et al., 2013). Ces techniques sont utilisées pour fournir une compréhension approfondie et plus

directe du comportement mécanique musculaire individuel (i.e. raideur passive) par rapport à des mesures plus globales fournies par des ergomètres isocinétiques (Hug et al., 2015). Plus précisément, la vitesse de l'onde de cisaillement mesurée par l'élastographie par ondes de cisaillement ultrasonores offre une opportunité unique de mesurer directement la raideur musculaire localisée. Ainsi, elle permet donc d'estimer la tension passive des muscles individuels lors de leur étirement (Hug et al., 2015; Nordez et al., 2017). Par conséquent, cette technique offre une perspective unique pour examiner les adaptations des tissus musculaires et non-musculaires en réponse aux interventions d'étirements lors des évaluations articulaires maximales de l'AAM. L'objectif de cette thèse était de mieux comprendre le rôle mécanique individuel des tissus musculaires et non-musculaires (i.e. nerf périphérique) qui peuvent influencer la limitation de l'AAM *in-vivo*. Quatre études ont été réalisées en utilisant la technique d'élastographie par ondes de cisaillement ultrasonore permettant d'évaluer la raideur passive des muscles et des nerfs individuellement afin de : **i)** analyser la raideur musculaire et le couple passif pendant l'évaluation de l'AAM sur différentes postures du membre inferieur (étude 1); **ii)** éterminer si les propriétés mécaniques des nerfs périphériques peuvent être évaluées *in-vivo* au cours d'un mouvement articulaire passif en utilisant l'élastographie par ondes de cisaillement ultrasonore (étude 2); **iii)** analyser s'il est possible d'étirer et de modifier la raideur des structures non-musculaires d'une part en l'absence de modifications mécaniques des muscles croisant l'articulation ; et d'autre part si ces adaptations sur les structures non-musculaires sont liées à des modifications de l'AAM (étude 3); **iv)** éterminer si les étirements tant aigus que chroniques des tissus nerveux ou des tissus musculaires modifient leurs propriétés mécaniques et contribuent ainsi aux adaptations de l'AAM (étude 4).

Cette thèse est divisée en cinq sections principales. Le chapitre 1 correspond à l'introduction. Le chapitre deux concerne la revue de la littérature sur la thématique. Le chapitre trois décrit la conception de la recherche et les méthodes communes aux quatre études. Les chapitres quatre et cinq sont respectivement relatifs aux études expérimentales et à la discussion générale.

La principale conclusion de cette thèse doctorale est que l'AAM de la cheville peut être limitée par la mécanique des structures musculaires ou non-musculaires et dépend fortement de la position du membre inférieur adoptée durant les tests articulaires. En outre, nos résultats indiquent que les nerfs périphériques peuvent jouer un rôle important sur l'AAM. De plus, cette thèse démontre que

l’étirement spécifique d’un tissu peut déclencher des adaptations mécaniques locales dans les structures individuelles et, par conséquent, induire des changements dans l’AAM. En effet, les étirements aigus et chroniques ciblant soit les muscles ou soit les nerfs ont induit des modifications locales des propriétés mécaniques respectives de ces tissus. Ainsi, les améliorations de l’AAM après les interventions d'étirements apparaissent spécifiques aux tissus les plus raides et corrélés à des modifications de leurs propriétés mécaniques.

Par conséquent, les résultats issus de ces travaux de thèse apportent une contribution significative et originale sur l'amélioration de la conception et de la prescription des programmes d'étirements individualisés. De plus, ils permettent d'interpréter de façon plus individuelle la portée clinique de l’étirement des structures musculaires ou non-musculaires dans les tests de l’AAM.

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***Mots clés :*** *amplitude articulaire maximale - raideur - élastographie ultrasonore par ondes de cisaillement - nerfs périphériques - muscle - couple passif – cheville*

*Chapter One*

# INTRODUCTION

Maximal range of motion (ROM) of a joint can be considered an integral part of human movement, and is defined as the motion available at any single synovial joint. It influences functional activities and athletic performances, may impact soft tissue injury risk, and changes notably with aging or immobilization (Akeson *et al.*, 1987; Trudel *et al.*, 1999; Mulholland & Wyss, 2001; Witvrouw *et al.*, 2003; Hemmerich *et al.*, 2006). Whilst the active maximal joint ROM is generated by voluntary muscle activation from the muscle-tendon unit (MTU) acting on the joint (contractility property provided by the myofilament interaction), the passive maximal joint ROM is the extent to which a joint can be moved in the absence of significant muscle activity (Gajdosik, 2001).

Traditionally, when assessed in a passive condition the limitation of maximal joint ROM has been attributed to two main entities: joints and muscles. For certain articular movements (e.g., knee extension, elbow extension) it is obvious that joint congruency and intra-articular structures, such as joint capsule, are the main restrictors of the maximal joint ROM. For other joints (e.g., hip, angle), it has been considered that maximal joint ROM is mainly restricted by the tension developed by MTUs being stretched during articular motion (Herbert, 1988; Magnusson, 1998; Gajdosik, 2001; Weppler & Magnusson, 2010). This passive muscle tension is dependent on both structural and mechanical properties of the muscle as well as surrounding myofascial layers, which have notable viscoelastic properties that provide most of the passive tension. However, there is current evidence suggesting that in some special configurations maximal joint ROM can be altered without triggering mechanical and structural adaptations within the MTU (Weppler & Magnusson, 2010; Freitas *et al.*, 2017; Nordez *et al.*, 2017).

During joint movement non-muscular tissues such as peripheral nerves and fasciae may also experience large changes in their length and tension; however, the influence of these non-muscular tissues on the maximal ROM available at a joint remains largely unexplored. For instance, significant decreases in maximal joint ROM have been found on the tested joint when changing the position of other distant and apparently non-musculoskeletal related joints (Gajdosik *et al.*, 1985; Mitchell *et al.*, 2008; Palmer *et al.*, 2015). Interestingly, it has been recently shown that myofascias and peripheral nerves, which are richly innervated with receptors that when strained trigger the central nervous system, may experience changes in their length during joint motion (Silva *et al.*, 2014; Cruz-Montecinos *et al.*, 2015). Thus, maximal joint ROM can be limited in

some body postures that notably stretch such non-muscular structures. However, there is currently a lack of scientific evidence to support, in a more direct way, whether or not the mechanical properties of non-muscular structures can affect maximal joint ROM.

Nerves have traditionally been suggested in reducing maximal joint ROM when tensioned, as observed in some clinical tests (Gajdosik *et al.*, 1985; Laessoe & Voigt, 2004; McHugh *et al.*, 2012; Andrade *et al.*, 2015). Concomitant with this finding, cadaver studies have demonstrated increases in strain in the tibial nerve at the tarsal tunnel, without affecting other musculoskeletal structures (Coppieters et al., 2006). However, there is a strong knowledge gap in the influence of nerve on the mechanical loading in the limitation of maximal joint ROM. Moreover, no previous study has assessed dynamically, using a non-invasive approach, the mechanical properties of peripheral nerves *in-vivo* as a response to joint movement.

In addition, global classical torque-angle assessments that are classically used to study the mechanical mechanisms underpinning the maximal joint ROM are not sufficiently sensitive to detect small mechanical changes in both muscular and non-muscular tissues. Furthermore, it has been suggested that non-muscular tissues may have a small contribution in global passive joint torque outcome (Nordez et al., 2017). Specifically, passive torque-angle relationships provide neither a direct quantification about individual tissue stiffness, nor information's about muscle geometry. These outcomes are crucial to have an exhaustive understanding of mechanical properties of both muscle and non-muscular structures underlying the limitation of maximal ROM at a given joint, notably after a stretch intervention.

The development of imaging techniques such as ultrasonography has allowed the evaluation of underlying architectures of the MTU in humans, both *in-vivo* and non-invasively. More recently, classic ultrasound imaging and shear wave elastography have emerged to assess the biologic tissue stiffness with good reliability (Bercoff et al., 2004; Gennisson et al., 2013). These techniques have been used to provide a deeper and more direct understanding of individual muscle mechanical behavior (i.e., passive stiffness) than more global measurements provided by isokinetic ergometers (Hug et al., 2015). Specifically, shear wave velocity measured by ultrasound shear wave elastography provides a unique opportunity to directly measure localized muscle stiffness, and thus to estimate the passive tension of individual muscles when being stretched (Hug et al., 2015; Nordez et al., 2017). Therefore, this technique offers a unique perspective to evaluate both

muscular and non-muscular structures during maximal joint ROM assessments, and to examine individual tissues adaptations as a response to stretch interventions.

Therefore, the intention of this thesis was to extend the knowledge the mechanical factors underpinning maximal ROM at a given joint by examining the individual mechanical role of both muscular and non-muscular tissues. To this end, individual adaptations to acute and chronic stretching stimuli were examined to reveal the specific mechanical contribution of these structures. This thesis provides a series of four studies that have addressed the issues raised above, with specific reference to maximal joint ROM of ankle joint, and to explore the factors affecting the maximal ankle ROM in dorsiflexion. Thus, an overall aim was to provide scientific evidence to support or refute mechanisms associated to both sensory- and mechanical-based theories and to analyze the individual contribution of non-muscular structures in the limitation of maximal ROM. This thesis is divided into four main sections. Chapter two reviews existing literature in the field. Chapter three describes the research design and methods common to the four studies. Chapters four refers to the experimental studies, and Chapter five provides general discussion.

*Chapter Two*

# LITERATURE REVIEW

# 1. Background

Both acute and chronic stretching are commonly performed in sports training and therapeutic interventions with the aim of improving maximal joint ROM and decrease tissues tension (Magnusson, 1998; Gajdosik, 2001; Weppler & Magnusson, 2010). Thus, the first and second parts of this review focus on the mechanical properties of the passive muscle and the mechanical adaptations following acute and chronic stretch interventions, respectively. In addition, other non-muscular tissues, such as the peripheral nerves, experience large changes in their length and tension during the traditional stretching maneuvers (Topp & Boyd, 2006; Silva et al., 2014; Nordez et al., 2017). However, the influence of these non-muscular tissues on the maximal joint ROM remains largely unexplored (Nordez et al., 2017). Thus, the third part of this chapter is focused on the mechanical properties of peripheral nerve and fascia and their respective adaptations to stretching. A comprehensive understanding of such tissues' adaptations to stretching is important to reveal their role in joint function and stretch amplitude (i.e., maximal ROM).

# 2. Passive joint and skeletal muscle mechanical properties

## 2.1. Introduction

*In-vivo* skeletal muscles elongate as a result of joint motion. From a given articular angle ($\theta_0$), MTU crossing the joint offers a tensile mechanical resistance to stretch. Passive stretching refers to the elongation of a MTU beyond $\theta_0$ by an external force. It is possible to characterize the passive mechanical properties of joints by studying the response of MTU to different passive stretching protocols with minimal or negligible muscle activity (Magnusson, 1998; Gajdosik, 2001), this being a key condition for a pure passive motion. Because this condition was respected throughout the experimental research conducted in this thesis, the neural factors are not considered in this review. Recent advances in imaging techniques (e.g., ultrasound shear wave elastography) have considerably increased the ability to characterize the passive mechanical properties of individual tissues of MTU in response to passive stretching (Hug *et al.*, 2015).

The following sections provide a brief overview of skeletal muscle passive mechanical properties. Specifically, it describes 1) basic concepts of isolated MTU mechanics; 2) how these structures can contribute to the limitation of maximal joint ROM; 3) the structures and mechanisms contributing to muscle's resistance in response to passive stretching; and 4) the current methods used to assess joint and skeletal muscle mechanical properties: torque-angle relationship and ultrasound shear wave elastography.

## 2.2. Basic concepts of isolated muscle-tendon mechanics

Several variables and theories are currently used in the field of biomechanics to study passive mechanical properties at both tissue (muscle) and joint levels. The purpose of this section is to review such variables and describe how they contribute to this doctoral research.

In the laboratory, it is possible to measure the change in muscle length and the passive tension that develops within the muscle by stretching isolated muscles (Gordon *et al.*, 1966; Taylor *et al.*, 1990; Davis *et al.*, 2003). Animal studies have used this length-tension relationship (Figure 1) to study the biomechanical behavior of MTU during passive lengthening until the failure point (Noonan *et al.*, 1994; Sun *et al.*, 1995). Interestingly, these experimental methods allow one to purely test the mechanical properties of MTUs (Herbert, 1988), particularly when isolated from other overlying and adjoining tissues such as skin, neurovascular structures, and *fasciae*. However, such invasive investigations are not possible in humans due to ethical reasons (Gajdosik, 2001).

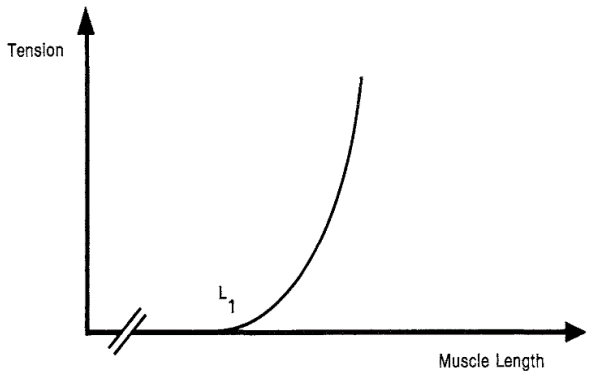


*Figure 1. Passive length-tension curve for skeletal muscle. $L_1$ is the length at which the muscle first develops tension (slack length). Figure reprinted from Herbert (1988); Copyright 1988, with permission from Elsevier Inc..*

When exploring mechanical properties of MTU, distinction between force-length and stress-strain characteristics is required. Passive tension-length relationships are calculated by measuring the tensile force applied and the resultant elongation of the tissue. Although similar, stress is calculated as the internal force divided by the cross-sectional area of the tissue and strain is the change in length divided by the initial length (Nubar, 1962). The elastic or Young's modulus is the slope of the relationship between stress and strain. Because the stress-strain curve relationship of biological tissues are nonlinear, the Young's modulus varies as a function of tissue stress. Classically, passive stiffness corresponds to a ratio between changes in passive force and changes in length.

The passive tension generated by a muscle increases exponentially as the muscle is lengthened beyond the slack length (Herbert, 1988; Lieber & Bodine-Fowler, 1993). This point ($L_1$ in Figure 1) corresponds to the initial length at which the muscle begins to resist the stretch. It is important to note that before the slack length there is no true tensioning of the fibers, being that this region is also considered as the muscle resting length. As the stretch of the muscle increases beyond the slack length, the muscle may exert a larger pull against the stretch (i.e., elastic region; between A and B in Figure 2) into the plastic region (between B and C in Figure 2), where the structure of the tissue is altered and the slope of the force-length relation changes (Enoka, 2015). For instance, if a tissue is stretched to point D, mechanical plastic changes will occur, resulting in a new resting length (point A') when the stretched tissue is released. The end of the plastic region occurs when the maximal passive resistance is reached. Stretching beyond the maximal length (point C in Figure 2) results in rupture of muscle fibers which commonly occurs at the level of the myotendinous junctions (Nikolaou *et al.*, 1987; Garrett *et al.*, 1989). Note that the physiological range of lengthening remains within the elastic range (between A and B in Figure 2). Therefore, the plastic behavior of the MTU will not be considered in this doctoral research.

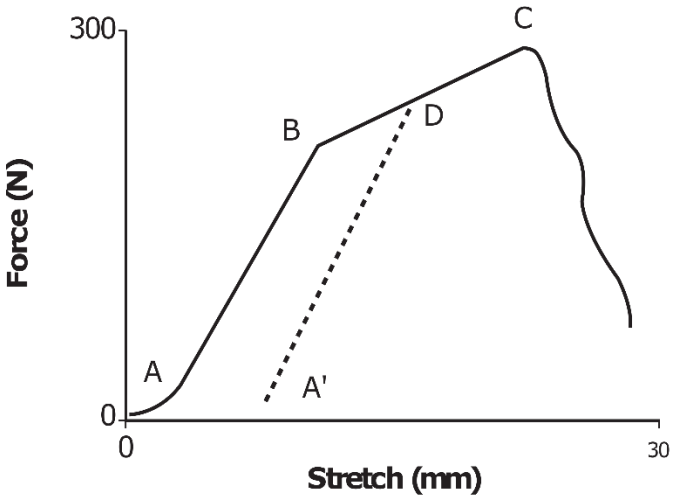


*Figure 2. An idealized force-length relation for connective tissue stretched beyond its elastic region (A-B) and into its plastic region (B-C). Region before the point A is defined as slack length range or toe region. Point C corresponds to the break/failure point of the tissue. Figure reprinted from Enoka (2015); Copyright 2015, with permission from Human Kinetics.*

Animal studies have shown that skeletal muscle, as most of biological tissues, have a viscoelastic behavior (Abbott & Lowy, 1956; Taylor *et al.*, 1990; Davis *et al.*, 2003), including both elastic and viscous properties. The viscoelasticity of MTU implies several mechanical characteristics. First, the force-length relationship is rate change-dependent (Figure 3).

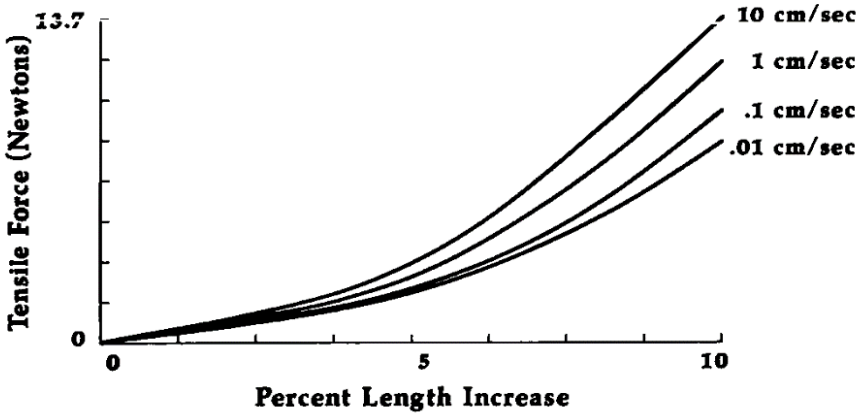


*Figure 3. Representative tensile force-length relationships at different stretch rates on a single muscle-tendon unit (tibialis anterior). Figure reprinted from Taylor et al. (1990); Copyright 1990, with permission from SAGE publishing.*

Second, the hysteresis is the loop of the loading-unloading cycle force-length or stress-strain relationships. The hysteresis area of stress-strain relationships represents the energy dissipated by

the tissues during the cycle. The normalized hysteresis can be used to infer about the dissipative properties related to the viscosity of the material being tested. Third, relaxation (Figure 4-A) is the decrease in tensile stress over time that occurs when the tissue under tensile stress is held at a fixed length (constant length), and creep (Figure 4-B) is the increase in tissue length as applied force is held constant (constant force). Both variables are helpful to characterize mechanically the MTUs, and to examine the acute adaptations of the MTU following a physical intervention such as stretching.

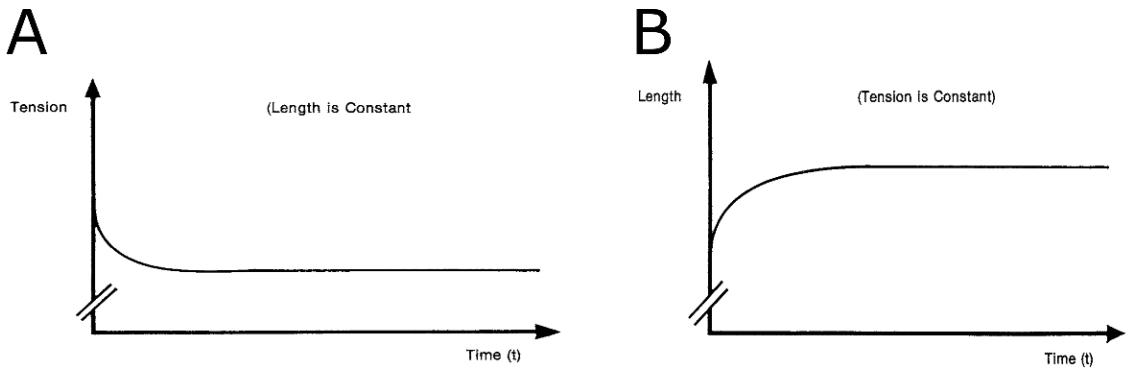


*Figure 4. Stress relaxation and creep.* ***A.*** *Stress relaxation: the muscle is stretched to a constant length and the tension in the muscle decreases over time to a new value.* ***B.*** *Creep: the muscle is stretched to a constant length and the length of the muscle increases over time to a new value. Figure reprinted from Herbert (1988); Copyright 1988, with permission from Elsevier Inc.*

## 2.3. Maximal passive joint ROM

When bony apposition is not the main limiting factor of articular mobility, passive maximal joint ROM is an important functional parameter to estimate the maximal length or muscle extensibility (Gajdosik, 2001; Nordez *et al.*, 2017). Specifically, the passive is the extent to which a joint can be moved in the absence of significant muscle activity (Gajdosik, 2001).

It has been widely used to evaluate the integrity of the MTU and, in more specific clinical contexts, to evaluate the status of intra-articular structures (Herbert, 1988; Weppler & Magnusson, 2010; Herbert *et al.*, 2015). In the laboratory, it is frequently assessed from classical torque-angle relationships measured using isokinetic dynamometers. However, first clinical investigations of human flexibility commonly used maximal joint ROM as a dependent and single variable (Norkin & White, 2009). Interestingly, clinical practice guidelines in orthopedics and physical therapy describe tests and instruments to assess maximal joint ROM and often point it as an indirect index

of muscle extensibility (Norkin & White, 2009; Porter, 2013). These "muscle length tests" were developed with the idea that there is an optimal range of MTU extensibility. If so, the maximal ROM of a joint should be highly dependent on the ability of MTU to adapt to the imposed mechanical stresses.

Excessive or insufficient MTU extensibility may result in increased or decreased joint ROM, respectively (Gajdosik, 2001; Weppler & Magnusson, 2010). For example, maximal joint ROM is significantly affected by contractures that are common complications of many neurological conditions including stroke, spinal cord injury, cerebral palsy, and traumatic brain injury; or musculoskeletal conditions and diseases including rheumatoid arthritis, burns, and surgery (Harvey & Herbert, 2002; Fergusson *et al.*, 2007). Reduced maximal joint ROM in these clinical populations is a major cause of joint function loss. It has been suggested that the increase in resistance to passive joint movement is the principal factor limiting joint mobility (Katalinic *et al.*, 2010). Accordingly, when used in an isolated form, maximal joint ROM itself does not clearly express an estimation of maximal length of the MTU or the magnitude of its extensibility (Weppler & Magnusson, 2010).

Importantly, however, when assessed *in-vivo* maximal passive ROM is also a measure of the individual's sensation at the endpoint of ROM. In respect to the literature, this point has commonly been termed the "sensory endpoint of pain" (Weppler & Magnusson, 2010) or maximal "stretch tolerance" (Halbertsma & Goeken, 1994; Halbertsma *et al.*, 1996). Although most of the guidelines of muscle length testing (i.e., goniometry) do not account for this parameter, it may have a significant role on the maximal ROM outcome (Norkin & White, 2009).

Moreover, its definition has been inconsistent among research studies, and recent efforts have been made to overcome this issue and standardize testing procedures (Freitas *et al.*, 2015c; Muanjai *et al.*, 2017). However, expressions related to either pain ("onset of pain", "tolerable pain", "just before the onset of pain", "point of discomfort") or stretch sensation thresholds ("strong of moderate pulling sensation", "sensation similar to a static stretching maneuver", "maximal tolerable passive torque threshold") have been frequently used to define maximal joint ROM testing (Magnusson *et al.*, 1995; Boyce & Brosky, 2008; McHugh *et al.*, 2012; Herda *et al.*, 2013; Matsuo *et al.*, 2013; Andrade *et al.*, 2016a; Muanjai *et al.*, 2017). This parameter can

influence the magnitude of stretching, and thus be a bias for interpretations of results from different studies.

As the pain may represent an intense stimuli, we contend that the "onset of pain" in the tissues being stretched should be an adequate instruction to standardize the evaluation of maximal joint ROM in humans. Although the relationship between pain and mechanical input from the tissues becomes less predictable in a population with chronic pain, also thought to have a reorganization of primary sensory and motor cortices, this should not be a major issue in healthy and asymptomatic populations (Moseley, 2008). Moreover, this doctoral research involved young, healthy, and pain-free volunteers. This instruction was considered in both maximal joint ROM assessments and stretch interventions performed in the present doctoral research to standardize both intensity and the magnitude of tissues' loading.

## 2.4. Passive joint torque-angle relationships

Because it is not possible to isolate a human muscle to obtain the passive force-length relationship *in-vivo*, isokinetic dynamometry has been thoroughly used over the last three decades to obtain torque-angle relationships (Figure 5). The passive tension (or passive torque) represents the global passive resistance of musculo-articular complex as a response to imposed articular motion.

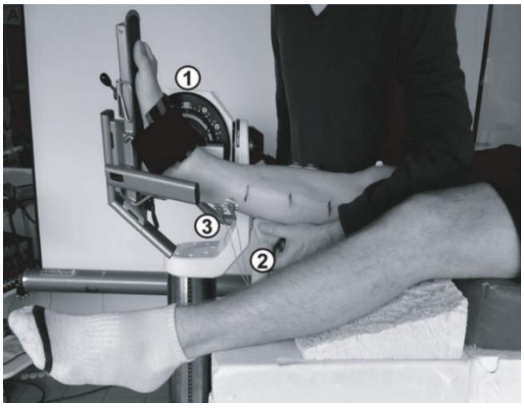


*Figure 5. Example of a testing setup to assess passive torque-angle of the ankle joint during passive rotations.* ***(1)*** *Dynamometer used to perform passive ankle rotations, measure the passive torque and estimate the ankle angle. Figure reprinted from Le Sant et al. (2017); Copyright 2017, with permission from John Wiley and Sons Inc.*

Joint passive-torque relationships have been used to examine the mechanical properties of muscle-tendon complex following acute and chronic stretching interventions in both healthy (Magnusson *et al.*, 1996c; Morse *et al.*, 2008; Blazevich *et al.*, 2014) and clinical populations (Harvey *et al.*, 2000; Bressel & McNair, 2002; Gao *et al.*, 2011), and to characterize the effects of either cyclic or static stretches on global muscle-tendon units (Nordez *et al.*, 2010b; Freitas *et al.*, 2015a). Hamstring and plantar flexors muscles are the most studied muscle groups (Magnusson, 1998; Weppler & Magnusson, 2010; Freitas *et al.*, 2017).

The assessment of passive mechanical properties in humans should be performed in the absence of muscle activity to minimize the presence of active forces on measurements (Magnusson, 1998; Gajdosik, 2001). As the level of muscle reflex activity may have a strong influence on the measurements of passive tension, during these assessments joints are commonly passively rotated at slow angular velocities (< 5°/s). Some authors have suggested that the level of muscle activity during passive elongation of muscle-tendon complex should be lower than 1-5% of the surface electromyography registered during maximal isometric voluntary contraction (McNair *et al.*, 2001; Gajdosik *et al.*, 2004; Portero & McNair, 2010). During passive elongation of the muscle-tendon complex at constant velocity, the resistive rotational force (i.e., torque) produced within the tissues can be measured using isokinetic dynamometry; and the joint angle can be estimated by aligning the center of rotation of the joint with the axis of the ergometer (Magnusson, 1998). Several mechanical outcomes can be derived from this assessment.

The slope of the torque-angle relationship (change in torque divided by change in position) has been calculated at a given joint angle (Kubo *et al.*, 2002; Guissard & Duchateau, 2004; Gajdosik *et al.*, 2007), and it has been defined as the overall joint passive stiffness of the muscle-tendon unit (Magnusson, 1998; McHugh *et al.*, 1998). Both passive torque and the slope of torque-angle relationship (Figure 6) have been used to examine muscle-tendon unit mechanical properties.

By analogy with force-length relationships, the viscosity of the global muscle-tendon unit has also been studied though torque-angle curves by: i) stretching tissues at different joint angle velocities (Nordez *et al.*, 2009); ii) during static phase of muscle-tendon unit stretch to measure the relaxation (Tian *et al.*, 2010; Freitas *et al.*, 2015a) and creep (Ryan *et al.*, 2008; Ryan *et al.*, 2010); and iii) calculating the normalized hysteresis area from the torque-angle relationship (Magnusson *et al.*, 1995; Magnusson, 1998; Nordez *et al.*, 2008a).

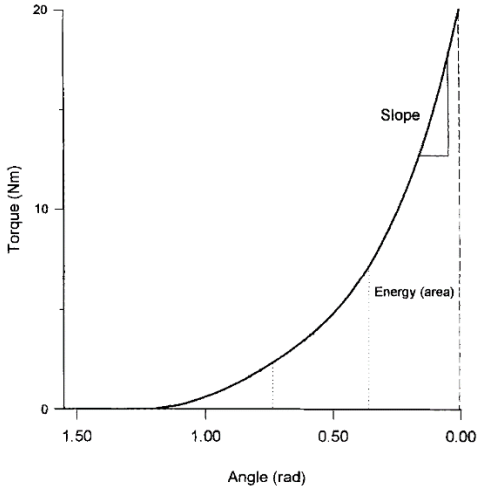


*Figure 6. Typical non-linear viscoelastic behavior of muscle-tendon unit during a stretching using a passive torque-angle assessment. Data corresponds to the dynamic phase of stretch maneuver, and represents an exponential increase in passive torque as a MTU is elongated (i.e., increase in joint angle). Joint stiffness is defined as the changes in torque (Nm) divided by the change in joint angle (radians) and expressed as the slope of the torque-angle curve. Figure reprinted from Magnusson (1998); Copyright 1998, with permission from John Wiley and Sons Inc.*

Importantly, maximal tolerated torque has been defined as the passive torque at the maximal joint ROM (Magnusson, 1998). This parameter has also been widely studied because it may indicate the human tolerance to stretch (Gajdosik et al., 2005; Blazevich et al., 2014; Nakamura et al., 2016). However, the end point of the movement (i.e., maximal joint angle) can be strongly influenced by factors such as pain, stretch tolerance, and reflex activation of the agonist muscle (Magnusson *et al.*, 1996c; Magnusson, 1998). Thus, it is important to instruct subjects to be relaxed during tests, and to define a consistent maximal joint ROM criteria (Portero & McNair, 2010; Weppler & Magnusson, 2010). Moreover, although it may represent the passive tension of muscle-tendon units being stretched, several studies have suggested that stretch tolerance and muscle extensibility can be limited by the stretching of non-muscular structures, such as the peripheral nerves (Laessoe & Voigt, 2004; McHugh *et al.*, 2012; Andrade *et al.*, 2015; Andrade *et al.*, 2016a).

Although the assessment of torque-angle is considered as an approximation of the MTU passive force-length relationship (Magnusson, 1998; Gajdosik, 2001), joint passive torque represents a global approach and thus it does not provide specific information about localized mechanical properties of the tissues lengthened during joint motion (including muscle, tendon, skin, nerves, fasciae, etc.) (Riemann *et al.*, 2001). Indeed, this has been considered to be a major limitation of this technique (Nordez *et al.*, 2017). As such, the understanding of individual

mechanical muscle function and the mechanisms underpinning tissue adaptations following acute of chronic solicitations remains largely unexplored.

Additionally, it remains unknown whether or not the mechanical loading of non-muscular tissues such as peripheral nerves, which may have little influence on passive torque, can restrict the maximal joint ROM (Nordez *et al.*, 2017). More direct experimental evidence is required to assess localized stiffness of individual structures among the musculo-articular complex, and to reveal whether or not muscle tissue responds to both acute and chronic stretching reflect joint mechanical adaptations.

## 2.5. Factors underlying the passive mechanical properties of MTU

Although MTU acts as a functional unit, it can be separated into the muscle belly and the tendons or aponeuroses connecting the muscle to the bone. Skeletal muscle is a hierarchical composite structure of contractile protein filaments (actin and myosin) and different layers of connective tissue: the epimysium surrounding the entire muscle belly, the perimysium encompassing bundles of muscle fibers, and the endomysium, which surrounds each individual muscle fiber (Stecco & Hammer, 2015). The muscle cell comprises a hierarchical structure with the sarcolemma (plasma membrane) surrounding the cell and sub-units termed myofibrils that contain several protein filaments including actin, myosin, titin, desmin, troponin and tropomyosin (Gray *et al.*, 2012).

A widely held view is that the passive mechanical properties of a musculo-articular complex are provided by several anatomical muscle components, but the relative contribution still remains largely unknown (Gajdosik, 2001). These components include contractile tissue, parallel elements to contractile tissues and series elements to the contractile tissues. Additionally, intra-articular structures may also have significant contribution and cannot be totally excluded.

### *2.5.1. Parallel elements to contractile tissues*

It has been suggest that most of the passive resistance produced as the MTU is stretched originates from the parallel elements to contractile tissues (Gajdosik, 2001). Although generally different in composition and structure, intramuscular connective tissue (i.e., epimysium,

perimysium and endomysium; Figure 7) is dominated by collagen, and not only ensures an organization into fascicles and fibers, but also contributes importantly to the force transmission (Aumailley & Gayraud, 1998; Purslow, 2002; Kjaer, 2004). Seven collagen types have been identified intramuscular connective tissue (types I, III, IV, V, VI, XII and XIV); however, types I and III are still considered the major fibrous collagen species in the reticular layer of the endomysium, epimysium and perimysium (Purslow, 2002). Collagen typically represents 1 to 10% of the dry weight of adult skeletal muscle, which is characterized by a small amount of fibers of elastin (typically less than 1% of muscle dry weight) (Purslow, 2010).

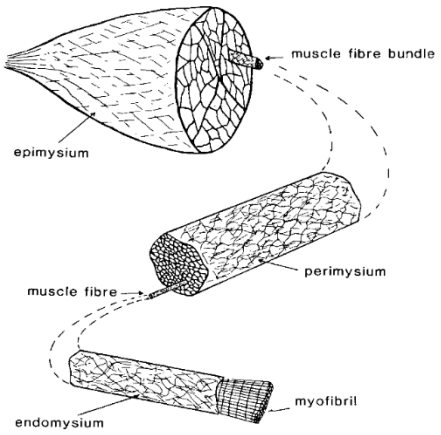


*Figure 7. Schematic diagram of the intramuscular connective tissue organization. Three distinct structures are shown. The whole muscle is surrounded by epimysium; muscle fibers bundles are separated by perimysium; and individual muscle fibers are separated by endomysium. Figure reprinted from Purslow (2002); Copyright 2002, with permission from Elsevier.*

In common with most soft biological tissues, these structures changes in length to accommodate the finite length changes of stretched muscle (Figure 8). For instance, the passive movement of the perimysium (the thicker structure) is viscoelastic, but it should not involve major reorientation movements of collagen fibers in a proteoglycan matrix (Purslow *et al.*, 1998). Rather, the viscoelastic pattern to stretch depends on relaxation processes within the collagen fibers themselves. Indeed, among the intramuscular connective tissues, the perimysium should be the major contributor to the passive tensile response of skeletal muscle to stretch (Purslow, 1989; Gajdosik, 2001; Purslow, 2002; Kjaer, 2004).

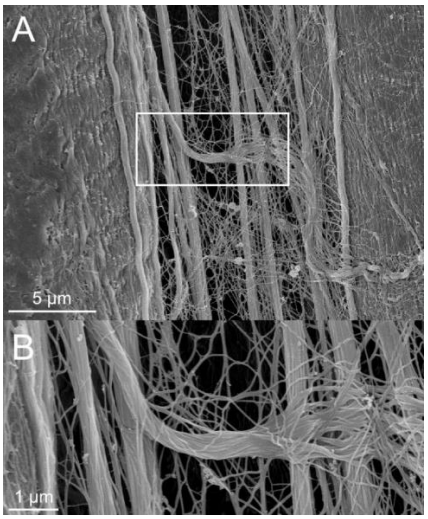


*Figure 8. Scanning electron micrograph of a stretched muscle (extensor digitorum longus of mouse). Muscle is stretched 30% beyond resting length showing longitudinally aligned perimysial collagen cables. (A) Stretched collagen cables separating two muscle fibers. The collagen cables are distinct from the muscle fiber surface. (B) A collagen cable becomes frayed as it traverses across collagen cables. Figure reprinted from Gillies and Lieber (2011); Copyright 2011, with permission from John Wiley & Sons, Inc.*

Furthermore, the presence of the non-contractile proteins such as titin and desmin has been noted to contribute to the passive tension of the sarcomere (Gajdosik, 2001). Both desmin and titin proteins contribute to the endosarcomeric cytoskeleton and exosarcomeric cytoskeleton, respectively (Wang *et al.*, 1993). They might contribute to the passive resistance, mainly when the muscle fiber is stretched beyond the actin and myosin overlap (Granzier & Pollack, 1985; Magid & Law, 1985). More recently, it was observed that the viscoelastic and passive force properties of isolated titin molecules are reflected in whole myofibril testing (Herzog *et al.*, 2012). Although it is considered as an important stabilizer of sarcomeres (Horowits & Podolsky, 1987), titin may also play a role as a producer of passive force (Granzier & Irving, 1995; Granzier *et al.*, 1997). Moreover, it has been shown that Type I muscle fibers have greater passive stiffness than Type II (Mutungi & Ranatunga, 1996, 1998). Interestingly, these differences may mostly reside in the different isoforms of titin within each muscle fiber. However, biomechanical findings of muscle fiber bundles and whole muscle suggest that titin plays only a minor role in passive load-bearing at the tissue level (Gillies & Lieber, 2011). For instance, the passive mechanical modulus of stretched fiber bundles is two-fold greater than single fibers (Ward *et al.*, 2009), strengthening therefore the contribution of perimysium and the other intramuscular connective tissue on the overall passive tension of the muscle.

### *2.5.1. Series elements to the contractile tissues*

Series elements to the contractile tissues comprise tendons, which are structures predominantly composed of water, which makes up 55-70% of total tendon weight. The majority of the tendon dry weight consists of collagens (60-85 %) (Thorpe & Screen, 2016). As the skeletal muscle, tendon has a multi-unit hierarchical structure composed of several connective sheaths surrounding distinct levels of organization, including collagen molecules, collagen fibrils, collagen fiber, and fascicles that run parallel to the tendon's long axis (Wang, 2006; Thorpe & Screen, 2016). Some authors have traditionally considered the muscle belly as the primary contributor of MTU to length-tension relationship during stretching (Gajdosik, 2001; Weppler & Magnusson, 2010) because they are much more compliant than the tendon or aponeurosis (Trestik & Lieber, 1993). As such, the latter elements have been considered as non-contributory to passive length-tension relationship of a stretched MTU (Gajdosik, 2001).

However, although muscle and tendon are anatomically distinct tissues, they interact in series to form a functional unit. Consequently, the mechanical response of MTU to passive stretching is not only dependent on muscle mechanical behavior, but also on the mechanical properties of adjoining tendon(s). The latter may undergo larger changes in length than muscles when joints are passively moved (Herbert *et al.*, 2002), and thus contribute to a large part of the total changes in length of MTU. For instance, it has been demonstrated that 67% of total change in muscle-tendon length during passive stretching is due to the lengthening of tendon, while elongation of muscle fascicles contributed with only 27% (Herbert *et al.*, 2002; Herbert *et al.*, 2011). Indeed, tendons should be intrinsically stiffer and less compliant materials than muscles (Herbert & Crosbie, 1997; Finni, 2006). For instance, Kubo *et al.* (2005) demonstrated that muscle fascicles of *gastrocnemius medialis* muscle strains about five times more than Achilles tendon and ten times more than aponeurosis during passive stretching. Consistent with these findings, Hoang *et al.* (2007) showed that tendons experienced much smaller strains (9%) than muscle fascicles (86%). Moreover, as muscles and tendons are organized in series, a change in passive tensile force in tendon will manifest in muscle, and vice-versa. Thus, although no direct measurements were performed on tendon structure in this doctoral research, the muscle stiffness was assessed directly using ultrasound shear wave elastography.

### *2.5.2. Contractile tissue and joint structures*

In regard to the contractile tissue, it still remains unclear whether the stable interactions between the actin and myosin contributes to the passive resistance to stretch in non-activated muscles as well as the arrangement of the sarcomere cytoskeleton contributes to the tensile resistance of MTU to stretch (Proske & Morgan, 1999; Gajdosik, 2001).

Finally, intra-articular components should also play a role in the passive resistance to stretch. For instance, in addition to passive tension of MTU, Riemann *et al.* (2001) attributed intra-articular structures (e.g., ligaments, joint capsule) and other structures spanning the joints (skin, ligaments, anterior and posterior surrounding compartment muscles) as potential contributory sources of passive tension. In accordance with this suggestion, Johns and Wright (1962) reported that resistance to passive movement arose primarily from the joint capsule (47%) and the muscles (41%). In the sequential resections of the tissues crossing the wrist joint that was performed, they demonstrated that the remainder of the resistance was provided by tendons (10%) and skin (2%).

## 2.6. Determination of muscle shear modulus using shear wave elastography

### *2.6.1. Elastography methods*

Elastography was first introduced at the beginning of the 1990s to quantitatively image Young's modulus (E) of biological tissues *in-vivo,* which is the physical parameter corresponding to the most relevant stiffness measure of a given material (Ophir *et al.*, 1991). The main advantage of this technology is the assessment of stiffness in localized areas of soft tissues. As such, it further provides additional and clinically relevant information (Ophir *et al.*, 1991; Gennisson *et al.*, 2013; Sigrist *et al.*, 2017).

The physical basis of elastography relies on using ultrasound to track the time-varying displacements in the tissue either due to a static stress or the propagation of the shear waves - i.e. dynamic stress (Bamber *et al.*, 2013; Gennisson *et al.*, 2013; Sigrist *et al.*, 2017). Thus, elastography methods can be classified according to two means of excitation: the quasi-static methods (qualitative) and the dynamic methods (quantitative**)** (Bamber *et al.*, 2013; Gennisson *et al.*, 2013).

Qualitative quasi-static techniques (e.g., strain elastography and strain-rate imaging) have limited application in musculoskeletal field, because they do not offer a quantitative value for the Young's modulus. Specifically, this technique allows for an estimation of strain associated to the application of a constant and unknown stress (Gennisson *et al.*, 2013; Klauser *et al.*, 2014; Hug *et al.*, 2015). Quantitative outcomes, though, are essential to either compare individuals and tissues, or evaluate the effectiveness of clinical interventions (Hug *et al.*, 2015).

Quantitative imaging requires the creation of a shear waves, which are produced by localized displacement induced by either short transient or oscillatory perturbation with a fixed frequency (Gennisson *et al.*, 2013). Shear waves are generated at low frequencies (10 to 2000Hz). Assuming a purely elastic medium, the propagation velocity of the shear waves is directly linked to the shear modulus of the tissue that is perturbed, as follows (1): $\mu = \rho V_s^2$, where $\rho$ is the estimated density of soft tissues ($\sim$1000 kg/m$^3$) and $V_s$ is the shear wave velocity. Accordingly, the stiffer the tissue, the faster the shear wave propagation. In quasi-incompressible medium like biological tissues (e.g., liver, breast), which are characterized by high-water content, and in isotropic homogenous medium, the Young's modulus (E) can be approximated as three times the shear modulus ($\mu$), as follows (2): $E = 3\,\mu$. The shear wave propagation velocity can thus be used to map the Young's modulus quantitatively. It is important to note, however, that skeletal muscle tissue exhibits anisotropic properties (Gennisson *et al.*, 2003; Gennisson *et al.*, 2010). Specifically, mechanical properties of skeletal muscle should be directionally dependent. Theoretically, this equation (Equation 2) could not be therefore applied to skeletal muscle tissues. Interestingly, using an in-vitro animal preparation, Eby *et al.* (2013) recently demonstrated that skeletal muscle shear modulus measured using shear wave elastography is strongly correlated to the Young's modulus assessed by traditional materials testing. This result indicates that assessment of muscle shear modulus provides an accurate mechanical characterization skeletal muscle tissue using elastography (Hug *et al.*, 2015).

Among the shear wave elastography techniques, both ultrasound elastography and magnetic resonance elastography have been used to directly measure the mechanical properties of muscle (Bensamoun *et al.*, 2008; Brandenburg *et al.*, 2014). Although magnetic resonance elastography offers excellent spatial resolution, this technique has several limitations that are similar to those in classical magnetic resonance imaging. Specifically, the acquisition time is long (from 15 s to

several minutes), which is a key limitation for studying the mechanical properties of MTU during dynamic conditions as passive joint motion and contractions (Bensamoun *et al.*, 2008).

Ultrasound shear wave elastography is often considered for its ease of use, real-time capability (i.e., instantaneous measurement of shear wave velocity), portability and low cost compared to magnetic resonance elastography. Over the last ten years, ultrasound shear wave elastography has seen considerable development. It can be utilized to produce two- or, as more recently introduced, three- and four-dimensional quantitative images of shear waves with a useful field of view (Gennisson *et al.*, 2015). Among the ultrasound shear wave techniques, Supersonic Shear wave Imagining (SSI) (Bercoff *et al.*, 2004) is currently the most widely used technique, because it provides an almost instantaneous measurement of shear wave velocity and an accurate imaging of tissues stiffness (Bamber *et al.*, 2013; Gennisson *et al.*, 2013; Brandenburg *et al.*, 2014).

Briefly, remote pressure or push is created by acoustic radiation force induced in a specific region of the medium (Bercoff *et al.*, 2004; Gennisson *et al.*, 2013). Then, this push is displaced at a supersonic velocity to induce a Mach cone to generate the propagation of shear wave along the direction of the probe (Figure 9).

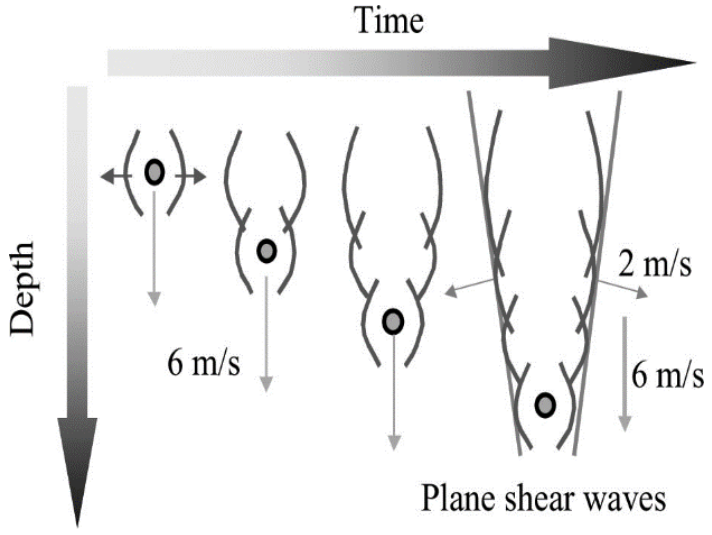


*Figure 9. Generation of the supersonic shear source. It is sequentially moved along the beam axis creating two intense- and plane shear waves. Figure reprinted from Bercoff et al. (2004); Copyright 2004, with permission from IEEE.*

Owing to ultrafast ultrasound imaging technology (Figure 10), the acquisition of the entire shear wave propagation field is then measured at a very high frame rate – up to 20 kHz (Bercoff

et al., 2004; Gennisson et al., 2013; Hug et al., 2015). Imaged shear wave velocity is then reconstructed using a time of flight algorithm (Bercoff et al., 2004; Gennisson et al., 2013). Finally, two-dimensional shear modulus imaging can be obtained using the Equation 1.

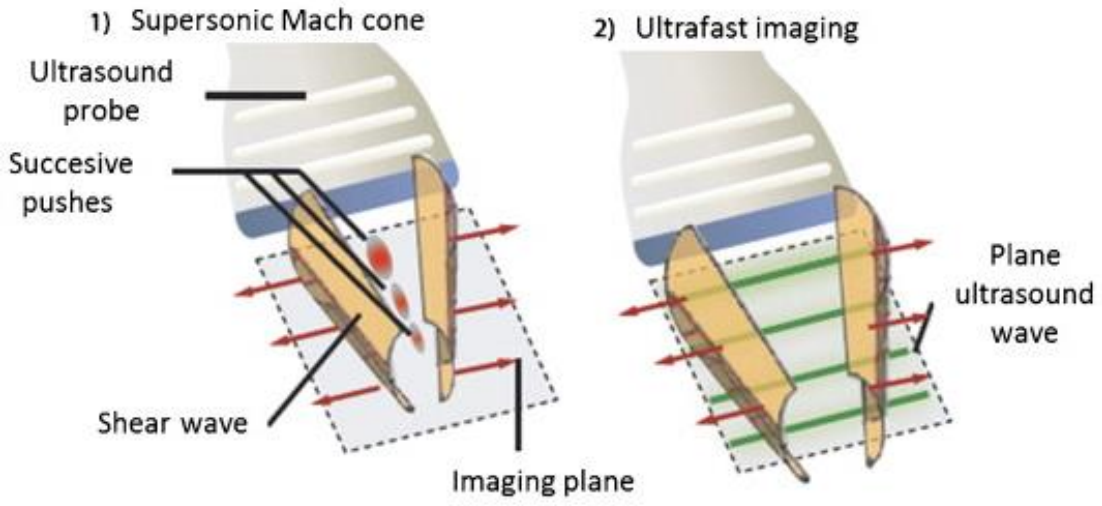


*Figure 10. "**1)** The ultrasounds are successively focused at different depths to create pushes by radiation pressure. The constructive interferences of the shear waves form a supersonic Mach cone (in which the speed of the source is greater than the speed of the generated wave) and a quasi-plane shear wave is created; **2)** The ultrasound machine then switches into an ultrafast imaging mode to follow the shear wave that is propagating through the medium." Figure reprinted from Gennisson et al. (2013); Copyright 2013, with permission from Elsevier.*

Although SSI has been widely used for diagnosis of breast cancer, liver fibrosis, and thyroid nodules, applications for the study of musculoskeletal system considerably increased over the last 5 years (Gennisson *et al.*, 2013; Hug *et al.*, 2015; Sigrist *et al.*, 2017). In human experiments the muscle extensibility and joint passive torque may be affected not only by intrinsic muscle-tendon related-features, but also by the relative contribution of intra-articular structures and passive non-muscular tissues, such as: skin peripheral nerves, *fasciae*, vascular structures (Riemann *et al.*, 2001; Nordez *et al.*, 2017). While the passive mechanical properties of a musculo-articular complex are classically estimated by passive torque-angle relationships as muscle-articular complex is passively elongated (Magnusson, 1998), the assessment of individual muscle mechanical properties *in-vivo* have remained a significant challenge for researchers.

Indeed, SSI represents a unique method to provide quantitative information about the individual mechanical behavior of both individual muscles (either mono- or bi-articular) and non-muscular structures acting around a given joint. In addition, quantifying musculoskeletal tissues

stiffness can provide the opportunity for a deeper understanding of changes in the biomechanical properties of these tissues with disease, rehabilitation and sport training (Hug *et al.*, 2015; Lima *et al.*, 2017).

### *2.6.2. Shear modulus as an assessment of muscle mechanical properties*

Several ultrasound shear wave elastography methods have been used to assess skeletal muscle stiffness with good temporal resolution. Since the beginning of the second decade of the twenty first century the use of this technique to study muscle biomechanics and localized tissue adaptations, in disease, rehabilitation or following sport training (e.g., stretching) has increased significantly (Hug *et al.*, 2015). This technique is a key measurement that is common to all research studies included in this doctoral research, and most of the studies presented in this section refers to the use of SSI in skeletal muscles during passive joint motion.

It has been shown that shear modulus measured using the SSI technique is a reliable measurement either at rest (Lacourpaille *et al.*, 2012) or during slow passive stretching (Maisetti *et al.*, 2012; Koo *et al.*, 2013; Freitas *et al.*, 2015a) conditions. Being an ultrasound-based technique, however, it is highly operator-dependent to obtain high reliable measurements. Here, good to very good inter-rater, intersession and intrasession reliability have been reported across several muscles (Lacourpaille *et al.*, 2012; Koo *et al.*, 2014; Dubois *et al.*, 2015; Freitas *et al.*, 2015a; Le Sant *et al.*, 2017). However, both muscle and tendon shear modulus may increase significantly with higher transducer pressures and measures should be performed with minimal pressure (Kot *et al.*, 2012). Interestingly, significant differences in shear wave velocity have not been observed in superficial median nerve when applying different levels of transducer pressure (Greening & Dilley, 2017). These particularities should be kept in mind to avoid acquisition errors and conflicting results between studies; and to obtain accurate, reliable, and repeatable measurements on musculoskeletal system tissues.

The quantification of the shear modulus provides a localized estimation of muscle passive stiffness. The use of this variable during passive joint motion offers a unique opportunity to characterize biomechanically the inter- and intra-muscle distribution of stiffness *in-vivo* (Figure 11) (Hirata *et al.*, 2015; Hirata *et al.*, 2016; Le Sant *et al.*, 2017).

For instance, by using this technique, Le Sant *et al.* (2017) showed a high variability of passive stiffness among lower leg muscles during passive ankle dorsiflexion. Most of the mono-articular dorsiflexors display low levels of passive stiffness during stretching when compared to gastrocnemii and soleus muscles. In addition, it has been shown that the passive stiffness within *triceps surae* muscles is not distributed homogenously (*gastrocnemius medialis* > *gastrocnemius lateralis* > soleus) (Hirata *et al.*, 2015; Hirata *et al.*, 2016; Le Sant *et al.*, 2017). Furthermore, the results of Le Sant *et al.* (2017) indicate that there is a proximo-distal distribution of the passive stiffness within localized muscles. They have effectively shown that proximal regions of *triceps surae* muscles are less stiff than the distal ones.

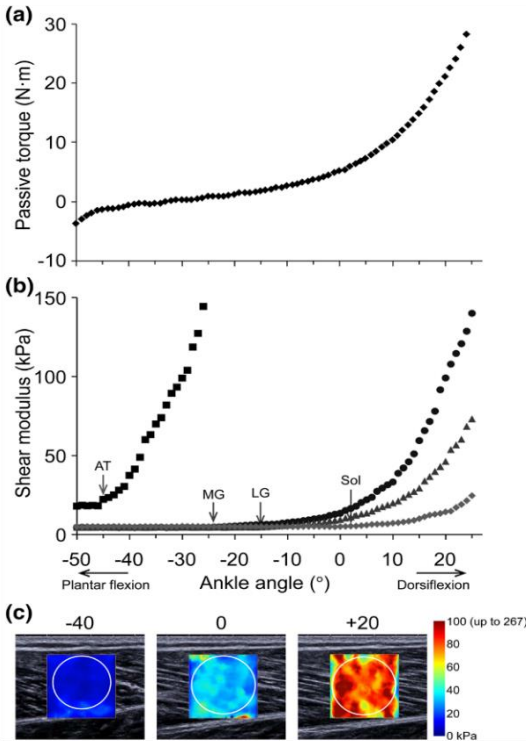


*Figure 11. Typical responses of ankle joint **(a)** and triceps surae responses to passive ankle rotation in dorsiflexion **(b)**; **(a)** Passive torque-angle relationship assessed using isokinetic dynamometer; **(b)** individual shear modulus of gastrocnemii (medialis – MG; lateralis – LG), soleus (SOL) and Achilles tendon (AT) assessed using ultrasound shear wave elastography (SSI); **(c)** typical examples of shear modulus measurements of LG obtained at −40°, 0°, and 20° of dorsiflexion angle. The colored region represents the shear modulus map. Figure reprinted from Hirata et al. (2016); Copyright 2016, with permission from Springer.*

Shear modulus assessment may help to determine the influence of each tissue (muscle, tendon, nerve, etc.) on maximal joint ROM improvements. These measurements can be performed either statically (Lacourpaille *et al.*, 2012; Akagi & Takahashi, 2014), at predefined joint angle, or

dynamically (along a joint range at angular velocities) (Hug *et al.*, 2013; Le Sant *et al.*, 2017). These stiffness comparisons are traditionally performed at a given angle or at the maximal ROM (point of maximal stretch tolerance). Interestingly, this elastography technique also allows the possibility to study the viscoelastic stress relaxation of tissues being stretch, which can be particularly useful to appreciate the effects of different percentage of MTU loading on individual tissues passive force loss over time (Freitas *et al.*, 2015a).

Furthermore, the measurements of shear modulus can be used to evaluate individual muscle stiffness adaptations to acute or chronic stretching, and whether or not such adaptations are homogenous intra- and inter-muscles (Akagi & Takahashi, 2014; Hirata *et al.*, 2015; Hirata *et al.*, 2016). For example, it is known that improvements in maximal joint ROM are induced by increases in the extensibility of soft tissues spanning joints.

### *2.6.3. Relationship between muscles shear modulus and passive force*

It has been shown that the shear modulus assessed by SSI is linearly related to muscle tension (Hug *et al.*, 2015). Until recently, only one elegant method (i.e. Hoang's model) could provide reliable passive force-length relationship of human muscle *in-vivo* (Hoang *et al.*, 2005; Nordez *et al.*, 2010a). This method uses muscle-tendon unit length estimated from cadaveric models and passive tension measured through torque-angle relationships (assessed through ergometers) to estimate the passive mechanical properties of bi-articular *gastrocnemius medialis* MTU at several different knee angles.

However, this is only valid for multi-joint muscles, and joint torque is the result of multiple structures. In an eloquent study using SSI, Maisetti *et al.* (2012) provided the first evidence to support that shear modulus provides an indirect estimation of passive muscle force (Figure 12). Specifically, they showed that both muscle length and shear modulus are strongly correlated to muscle passive force estimated by the Hoang's model (Hoang *et al.*, 2005). Although this musculoskeletal model predicts passive muscle force using many empirical assumptions, an *ex-vivo* study has directly confirmed such results (Koo *et al.*, 2013). This latter study used *gastronomies pars externus* and *tibialis anterior* chicken muscles to demonstrate, *in-vitro*, that the relationship between shear modulus assessed using SSI and passive muscle force are highly linear

(mean $R^2$=0.988; range: 0.974 and 0.994). This result highlights the idea that shear modulus assessed using SSI may be used as an indirect measure of changes in passive muscle force.

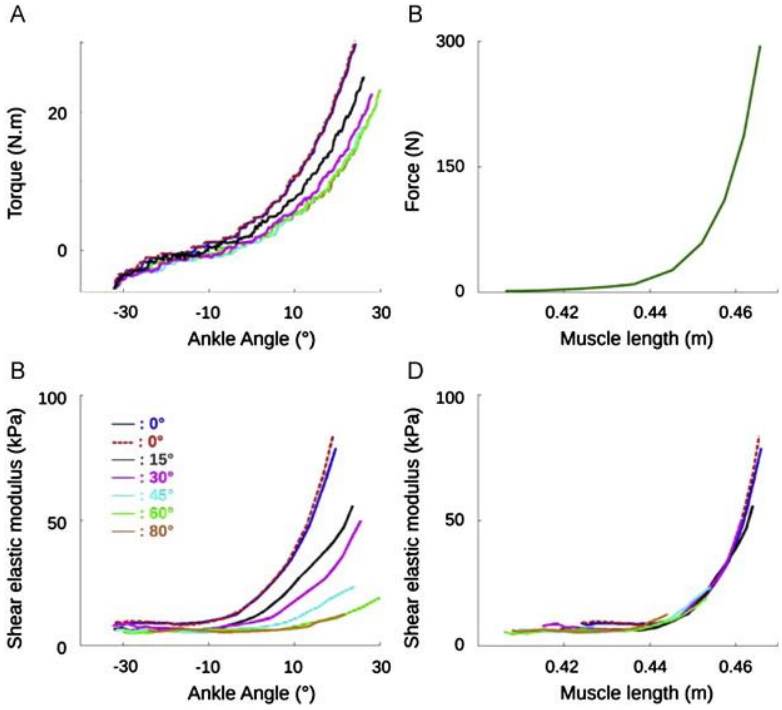


*Figure 12. (Colour outline represents different testing at various knee angles: 0°=fully extended). (A) Traditional passive torque-ankle angle curves during ankle dorsiflexion using isokinetic dynamometer; (B) Muscle force–length relationship obtained using the Hoang's model; (C) Relationship between shear modulus assessed by ultrasound shear wave elastography (SSI) and ankle angle; (D) relationship between shear modulus and muscle length (assessed by classic ultrasound B-mode image) during passive dorsiflexion. -30° = plantar flexion and 30° = dorsiflexion. Figure reprinted from Maisetti et al. (2012); Copyright 2012, with permission from Elsevier.*

Additionally, muscle slack angle can be estimated (Hug *et al.*, 2013; Hirata *et al.*, 2017; Le Sant *et al.*, 2017). This parameter is related to the muscle slack length that is an important parameter for musculoskeletal modeling (Hoang *et al.*, 2005; Nordez *et al.*, 2010a). Taking advantage of a strong linear relationship between shear modulus and passive muscle force along the long axis of a muscle, the slack length of individual muscles can now be evaluated in a more direct way by SSI. It is defined as the angle beyond which shear modulus is increased, and may provide a better understanding of MTU mechanical behavior, before and after clinical interventions, in musculoskeletal and neurological conditions that are commonly managed with stretch. However, because SSI has been increasingly used for assessing muscle shear modulus associated with age, musculoskeletal, and neurological conditions, Koo and Hug (2015) derived a

mathematical model from a widely-accepted Hill-type passive force-length relationship to understand the physiological meaning of resting shear modulus of skeletal muscles under passive stretching. This study confirmed that the resting shear modulus under passive stretching has a strong linear relationship with passive muscle force. However, the shear modulus of a resting muscle at its slack length and the rate of increase in shear modulus per unit passive force are functions of mechanical, material, and architectural properties of skeletal muscles. Therefore, shear modulus measurements should be interpreted with caution, particularly under clinical conditions or contexts in which architectural properties could be altered.

## 3. Joint and skeletal muscle mechanical adaptations to stretching

Although there is a wide range of stretching techniques, static, dynamic and proprioceptive neuromuscular facilitation (PNF) are the most frequently described in the literature (Behm *et al.*, 2016a). Among these three techniques, traditional static stretching is the most common (Page, 2012). The static technique consists in passively elongate the MTU to a given length and the maintained over a specific time. The effects of stretching can be divided in acute or chronic (usually defined as intervention protocols longer than 3 weeks). Moreover, depending on the assessment method used, mechanical responses to stretch interventions can be observed at two levels: joint (passive torque-angle relationships) and muscle tissue (shear modulus assessed by ultrasound shear wave elastography). In addition, stretching can induce changes in muscle geometry, which may be also reflected in maximal joint ROM. Traditionally, muscle geometry is assessed using ultrasound B-mode imaging.

Because this doctoral research is mainly focused on passive biomechanical properties of musculoskeletal system structures contributing to limitations in maximal passive ROM, the following sections section will briefly discuss the acute and chronic effects of static stretching interventions on MTU structure, including both geometry (fascicle length and angle, muscle cross-sectional area and volume) and mechanical properties (joint passive torque, and individual passive muscle stiffness). In contrast, neuromechanical adaptations (e.g., volitional muscle activation or motoneuron pool excitability) were not considered. However, they should have a limited impact in ROM changes after stretching (Blazevich *et al.*, 2014).

## 3.1. Acute adaptations

Acute effects of static stretching have been extensively studied at a joint level (McHugh & Cosgrave, 2010), and more recently at a muscle tissue level. Acute stretch regimens may alter the mechanical properties of muscle-tendon complex and, consequently, increase the maximal ROM at a given joint. For example, systematic reviews describing the acute effects of static stretching on maximal ROM have reported that static stretching could increase notably the ankle dorsiflexion (Radford *et al.*, 2006) and knee extension (Decoster *et al.*, 2005). Viscoelastic deformation and changes in muscle architecture (resting or during joint motion) have been proposed to explain increased maximal ROM following an acute stretching of MTU.

### *3.1.1. Mechanical factors underpinning changes in maximal ROM*

Muscle viscoelasticity has been considered to be predominately responsible for the increases in maximal joint ROM following acute stretching (Weppler & Magnusson, 2010). The passive torque-angle relationships have been traditionally used to evaluate these mechanical effects, at the joint level (Magnusson, 1998; Gajdosik, 2001; Weppler & Magnusson, 2010).

It is the consensus that relative stiffness of the musculo-articular complex decreases following an acute bout of static stretch (Magnusson, 1998; Weppler & Magnusson, 2010; Behm *et al.*, 2016a). Notably, these studies have observed a reduction in passive joint torque at a given range of joint angle, which might suggest a decreased stiffness of the stretched tissues (Kubo *et al.*, 2002; Morse *et al.*, 2008).

Additionally, acute stretching increases the capacity of MTU to tolerate loading prior to stretch termination (i.e., stretch tolerance) (Magnusson *et al.*, 1996b). Traditionally, these mechanical changes induce a shift to the right in the passive-torque angle curve following a static stretch (Weppler & Magnusson, 2010). In an extensive review about acute stretch effects on joint mechanical properties, McHugh and Cosgrave (2010) have concluded that a higher stretch duration may be related to a greater decline in passive torque. More recently, Freitas *et al.* (2015d) observed that a higher stretch duration with a low intensity should be a crucial factor for a reduction in passive torque, whereas a higher stretch intensity should be more effective for a greater gain in maximal ROM. The substantial differences in study methodology (i.e., duration, intensity, muscle

group, subject demographics) may therefore limit the understanding of these mechanisms to increases in maximal ROM after acute static stretching. Collectively, data suggest that longer stretch durations (> 5 min.) consistently alters the mechanical properties of MTU (McHugh & Cosgrave, 2010; Freitas *et al.*, 2015a; Nakamura *et al.*, 2015; Hirata *et al.*, 2016). In contrast, most of the studies that reported no significant changes in passive torque and/or muscle stiffness (slope of torque-angle relationship) have used stretching duration's inferior to 45 s (Muir *et al.*, 1999; Nakamura *et al.*, 2015). Furthermore, transient stress induced by acute stretch interventions may not be sufficient to trigger lasting mechanical adaptations. Accordingly, it has been suggested that the decrease in passive MTU stiffness observed after an acute stretch intervention may disappear within 10 to 60 min. after stretching, this being highly dependent on the time under stretching (Magnusson *et al.*, 1996a; Ryan *et al.*, 2008; Kay & Blazevich, 2009; Mizuno *et al.*, 2013a; Taniguchi *et al.*, 2015).

However, there has also been experimental evidence demonstrating that both maximal ROM and the overall stretch tolerance can be increased after a bout of MTU static stretch in the absence of changes in passive torque-angle relationship at a given angle (Halbertsma *et al.*, 1996; Magnusson *et al.*, 1998; Muir *et al.*, 1999). Thus, these studies have attributed the increased ROM following the stretch to an increased stretch tolerance, rather than changes it MTU mechanical properties. For instance, (Magnusson *et al.*, 1998) observed a single 90 s static stretching of hamstring increased maximal joint ROM and decreased muscle stiffness (slope of torque-angle relationship). Surprisingly, however, 10 min. after the stretching they still observed an increased maximal joint ROM yet in the absence of stiffness adaptations in MTU. Overall, these results suggest that acute stretching can induce increases in stretch tolerance while the viscoelastic properties of the muscle remain unaltered.

Because it is difficult to determine alterations in stiffness of individual muscular and non-muscular structures based on torque-angle relationship, ultrasound shear wave elastography was recently introduced to study the mechanical effects (i.e., changes in stiffness) of acute stretching interventions in individualized components of MTU (Freitas *et al.*, 2015a; Hirata *et al.*, 2017). Interestingly, Freitas *et al.* (2015a) showed that the ankle torque response during and following a 10 min. static stretching of plantar flexors does not reflect the changes in stiffness of *gastrocnemius medialis* muscle. This suggests that mechanical effects of acute stretching may vary among

muscles acting on the same joint. Moreover, they also observed that muscle stiffness is reduced only when sufficient mechanical stress is imposed on the muscle during stretching (i.e., stretch intensity).

As described above, stiffness mapping studies have taken advantage of shear wave elastography to demonstrate that lower leg muscles display a high variability of passive stiffness during passive ankle dorsiflexion (Hirata *et al.*, 2015; Taniguchi *et al.*, 2015; Hirata *et al.*, 2016; Le Sant *et al.*, 2017). This mechanical loading variability may contribute to the individual adaptations across muscles crossing the same joint after an acute stretch intervention. Thus, Hirata *et al.* (2016) observed that following a 5 min. static stretching of the *triceps surae* only decreased the passive stiffness of the stiffer *gastrocnemius medialis* without changes in *soleus* and *gastrocnemius lateralis*. A large part of this reduction was attributed to an increase in the slack length (estimated in the study from the slack angle of the shear modulus-angle relationship). Using the same methodology and an identical acute intervention, the same team showed that the increase in dorsiflexion ROM and decrease in ankle passive torque observed after the stretching can be explained by both the shift in slack angle (to a more dorsiflexed position) and a reduction in passive fascicle stiffness (Hirata *et al.*, 2017). Notably, the decrease in passive fascicle stiffness (estimated from the relationship between the fascicle length and the shear modulus) may have a greater effect on the decrease in passive torque at the latter part of ROM (longer fascicle length).

### *3.1.2. Changes in muscle architecture*

Although it is the consensus that muscle elongation for a given stretch intensity is mainly governed by MTU stiffness, structural changes in muscle architecture might also contribute to the increase in maximal ROM after acute stretching. However, most of studies have observed that angle-specific fascicle length (Morse *et al.*, 2008; Nakamura *et al.*, 2011; Ce *et al.*, 2015; Freitas *et al.*, 2015a; Opplert *et al.*, 2016; Hirata *et al.*, 2017) and pennation angle (Morse *et al.*, 2008; Ce *et al.*, 2015; Opplert *et al.*, 2016; Hirata *et al.*, 2017) remain unchanged following an acute stretch intervention. Interestingly, it was recently observed that total fascicle elongation of *gastrocnemius medialis* and *rectus femoris* increased immediately after an acute bout of static stretching, although no changes were reported in a resting position (Bouvier *et al.*, 2017). Additionally, it has been shown that changes in maximal ROM could also be related to an higher extensibility of MTU

induced by an increased displacement in both myotendinous junction (Nakamura *et al.*, 2011) and connective tissues of MTU (including tendon) (Kubo *et al.*, 2001; Morse *et al.*, 2008). In contrast, though, Mizuno *et al.* (2013b) and Freitas *et al.* (2016) did not observe motion changes in these structures after an acute stretching protocol.

## 3.2. Chronic adaptations

It has been widely demonstrated that chronic stretching induces a significant increase in maximal ROM (Young *et al.*, 2013; Medeiros *et al.*, 2016; Freitas *et al.*, 2017). Some of these studies have also examined the effects of long-term stretch interventions on mechanical (at both joint and muscle tissue levels) and structural adaptations of MTU, to determine the biomechanical and physiological factors that could explain the chronic adaptations in joint ROM (Freitas *et al.*, 2017).

### *3.2.1. Mechanical adaptations*

It is a long held belief that the increase in maximal ROM observed following chronic stretching induces a greater capacity of MTU to tolerate passive force (Freitas *et al.*, 2017). Most of the studies that assessed adaptations in mechanical properties have used passive torque-angle relationships. It has been shown that maximal tolerated passive torque increases significantly after a chronic stretch regimen (Halbertsma & Goeken, 1994; Reid & McNair, 2004; Gajdosik *et al.*, 2005; LaRoche & Connolly, 2006; Gajdosik *et al.*, 2007; Law *et al.*, 2009; Ylinen *et al.*, 2009; Ben & Harvey, 2010; Reid & McNair, 2010; Blazevich *et al.*, 2014; Nakamura *et al.*, 2016). Only two studies did not find significant pre- to post-stretching changes in this variable (Chan *et al.*, 2001; Guissard & Duchateau, 2004).

In addition, only two studies show a significant decrease in passive joint torque at a given angle after a chronic stretch training (Mahieu *et al.*, 2007; Nakamura *et al.*, 2012) that could mechanically explain the improvements in maximal ROM (Figure 13). Other studies, though, have only reported a tendency for a reduction between 1% and 10%, but without statistically significant changes (Mahieu *et al.*, 2009; Blazevich *et al.*, 2014; Konrad & Tilp, 2014b, a; Konrad *et al.*,

2015). In contrast, two studies have shown an increase in passive torque at a given angle (Gajdosik *et al.*, 2005; Gajdosik *et al.*, 2007).

**Passive torque at a given angle**

| Study name | *Intervention* | Std diff in means | Lower limit | Upper limit | p-value |
|---|---|---|---|---|---|
| Konrad & Tilp (2014a) | 6 wks, static 600s/wk | -0.06 | -0.74 | 0.61 | 0.85 |
| Gajdosik (2007) | 6wks, static 750s/wk | 2.58 | 0.89 | 4.28 | 0.00 |
| Mahieu et al. (2009) | 6wks, PNF 1260s/wk | 0.02 | -0.48 | 0.52 | 0.93 |
| Mahieu et al. (2006) | 6wks, static 700s/wk | -0.11 | -0.61 | 0.39 | 0.67 |
| Mahieu et al. (2006) | 6wks, dynamic 700s/wk | 0.31 | -0.25 | 0.87 | 0.28 |
| Nakamura et al (2012) | 4 wks, static 840s/wk | -5.63 | -7.69 | -3.57 | 0.00 |
| Konrad & Tilp (2014b) | 6 wks, dynamic 600s/wk | -0.07 | -0.74 | 0.60 | 0.83 |
| Konrad et al (2014) | 6 wks, PNF 720s/wk | 0.09 | -0.61 | 0.80 | 0.80 |
| Gajdosik (2005) | 6 wks, static 450s/wk | 1.03 | 0.07 | 1.98 | 0.04 |
| Blazevich et al. (2014) | 3 wks, static 1680/wk | -0.51 | -1.37 | 0.34 | 0.24 |
| | Overall ($I^2$=80%) | -0.04 | -0.57 | 0.49 | 0.89 |

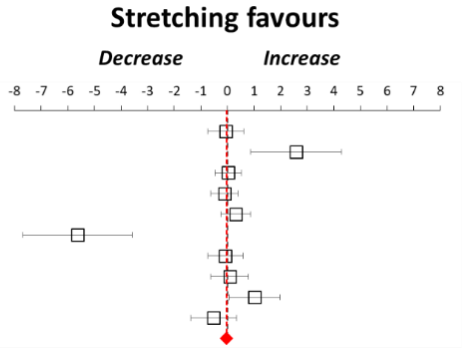


*Figure 13. Forest plot of the effect sizes and 95% CI of the changes in passive torque at a given angle.CI, confidence interval; Std diff, standardized difference. Figure reprinted from Freitas et al. (2017); Copyright 2017, with permission from John Wiley & Sons, Inc.*

Interestingly, data from the slope of torque-angle curve (i.e., relative stiffness) are more equivocal (Figure 14). Several studies have observed either increased (Reid & McNair, 2004; Gajdosik *et al.*, 2005; Reid & McNair, 2010) or reduced (Guissard & Duchateau, 2004; Marshall *et al.*, 2011) stiffness after chronic stretching. In accordance with these findings, muscle stiffness estimated from both muscle-tendon junction displacement of specific muscle assessed by ultrasound B-mode and passive joint torque (normalized to moment arm and cross sectional area) seem to remain unchanged after a chronic stretch regimen (Konrad & Tilp, 2014b, a; Konrad *et al.*, 2015), whereas a couple of studies reported a significant reduction (Blazevich *et al.*, 2014; Nakamura *et al.*, 2016).

**Slope of torque-angle curve**

| Study name | *Intervention* | Std diff in means | Lower limit | Upper limit | p-value |
|---|---|---|---|---|---|
| Konrad & Tilp (2014a) | 6 wks, static 600s/wk | 0.55 | -0.14 | 1.24 | 0.12 |
| Gajdosik et al. (2007) | 5 wks, static 750s/wk | 1.08 | -0.27 | 2.43 | 0.12 |
| Kubo et al. (2002) | 2.86 wks, static 3150s/wk | -0.92 | -1.95 | 0.11 | 0.08 |
| Guissard et al. (2004) | 6 wks, static 3000s/wk | -5.09 | -6.74 | -3.44 | 0.00 |
| Marshall et al. (2011) | 4 wks, static 1800s/wk | -0.91 | -1.79 | -0.03 | 0.04 |
| Konrad & Tilp (2014b) | 6 wks, dynamic 600s/wk | 0.51 | -0.17 | 1.19 | 0.14 |
| Konrad et al (2014) | 6 wks, PNF 720s/wk | 0.14 | -0.57 | 0.84 | 0.71 |
| Laroche (2006) | 6 wks, dynamic 900s/wk | -0.43 | -1.34 | 0.48 | 0.36 |
| Laroche (2006) | 6 wks, static 900s/wk | -0.47 | -1.36 | 0.42 | 0.30 |
| Reid et al (2011) | 6 wks, static 900s/wk | 0.90 | 0.24 | 1.56 | 0.01 |
| Akagi et al (2014) | 5 wks, static 2160s/wk | -0.21 | -0.85 | 0.43 | 0.52 |
| | Overall ($I^2$=83.5%) | -0.30 | -0.92 | 0.32 | 0.345 |

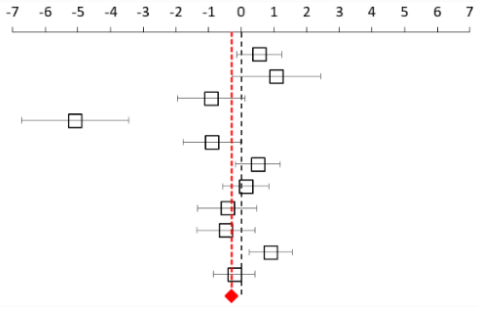


*Figure 14. Forest plot of the effect sizes and 95% CI of the changes in the slope of torque-angle curve.CI, confidence interval; Std diff, standardized difference. Figure reprinted from Freitas et al. (2017); Copyright 2017, with permission from John Wiley & Sons.*

More recently, two studies examined more directly the muscle stiffness adaptations by using ultrasound shear wave elastography to assess local changes in muscle stiffness (Akagi & Takahashi, 2014; Ichihashi *et al.*, 2016). For example, Akagi and Takahashi (2014) reported an homogeneous decrease in *gastrocnemius medialis* and lateralis stiffness after a 5-week static stretching of *triceps surae* that could explain the increased maximal ROM. However, joint torque at a given angle remained unchanged suggesting that changes in individual *gastrocnemii* stiffness did not reflect adaptations in global passive resistance to stretch of musculo-articular complex. An absence of mechanical changes in soleus, deep plantar flexors, and non-muscular structures contributing to joint passive torque could, however, explain these incongruous results. In addition, measurements were performed in a transverse plane of muscle, this being inappropriate to track accurately the propagation of shear waves (Gennisson *et al.*, 2010). Interestingly, Ichihashi *et al.* (2016) found a stiffness decrease across all hamstring muscles after a 4 weeks of hamstring stretch training. Here, measurements were performed along the main axis of the muscle fascicles.

Concomitant with a recent systematic review and meta-analysis performed by our team (Freitas *et al.*, 2017), these results suggest that chronic stretching inferior to 8 weeks seems to not induce mechanical adaptations at both joint and muscle levels, and thus could not explain the improvements in joint ROM. Therefore, chronic stretching may mainly induce alterations in the sensory system. Both loading (time under stretching) and intensity applied to MTU may not be sufficient to induce significant mechanical changes in MTU tissues. The effects of longer interventions (> 8 weeks) in individual muscular structures remain to be analyzed. Interestingly, this review shows a high variability among the changes in several mechanical and architectural variables after stretch interventions.

### *3.2.2. Changes in muscle architecture*

Animal studies indicate that soft tissues undergo structural adaptations in response to intensive and long-term (4 weeks) sustained stretching (Tabary *et al.*, 1972; Goldspink *et al.*, 1974; Williams & Goldspink, 1976). Specifically, these studies demonstrate that the increased number of muscle sarcomeres that are in series is dependent on the lengthening degree. However, these studies have used plaster casts to set a specific articular angle so that the muscles could be maintained in the

lengthened position for the whole duration of the chronic protocol. Thus, the stretching protocols used in these animal studies differ significantly from those perform in humans.

In humans, chronic stretching may induce trivial effects on fascicle length and fascicle angle (Freitas *et al.*, 2017). Most of the studies assessing muscle fascicle length have not observed length adaptations following classical stretch training with rest intervals (Nakamura *et al.*, 2012; Blazevich *et al.*, 2014; Konrad & Tilp, 2014b, a; e Lima *et al.*, 2015; Konrad *et al.*, 2015). Interestingly, Freitas and Mil-Homens (2015) reported a significant increase in *biceps femoris* fascicle length after a 8-week high-intensity (non-rest interval) stretch protocol. These results suggest that the use of a higher stretch intensity, favored by the absence of rest between stretch repetitions (Freitas *et al.*, 2015b), can be important to trigger fascicle length adaptations. However, such results should be interpreted with caution since a very small sample size was used in both control and experimental groups (n=5).

Concomitant with the lack of fiber length adaptations, no changes in fascicle angle have been observed across long-term stretch interventions (Konrad & Tilp, 2014b, a; e Lima *et al.*, 2015; Freitas & Mil-Homens, 2015; Konrad *et al.*, 2015).

## 3.3. Theories for increasing maximal joint ROM

The maximal ROM of a joint is an important functional parameter to estimate the maximal muscle length or muscle extensibility. It is well documented that both acute and chronic stretching induces an increase in maximal passive ROM (Weppler & Magnusson, 2010; Nakamura *et al.*, 2015; Behm *et al.*, 2016a; Freitas *et al.*, 2017). Hence, muscle extensibility might increases as a result of increased passive tension applied to MTU – i.e., muscle stretches further (Blazevich *et al.*, 2014). Two main theories have been proposed to explain the increases in joint maximal ROM following a static stretch regimen: mechanical- or sensory-based theories (Weppler & Magnusson, 2010; Nordez *et al.*, 2017).

Classically, increases in maximal ROM following stretch interventions have been attributed to decreases in both joint and muscle stiffness or changes in muscle architecture: mechanical-based theory (Figure 15). However, evidence for such mechanical adaptations have remained inconsistent (Weppler & Magnusson, 2010; Freitas *et al.*, 2017; Nordez *et al.*, 2017).

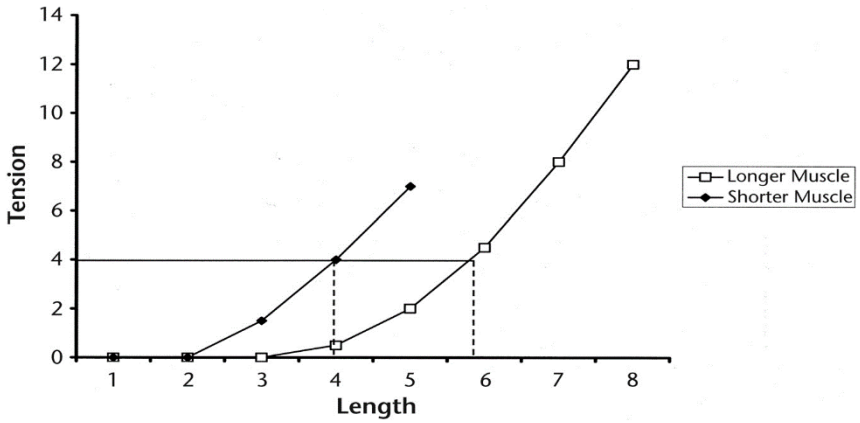


*Figure 15. Mechanical theory to explain the maximal ROM adaptations after chronic stretching, assuming that torque-angle reflects the muscle passive length-tension. Joint mechanical adaptations should be expected to occur at two different lengths in the torque-angle relationship. Shorter and longer muscles correspond to pre and post chronic stretching, respectively. After chronic stretching, for the same muscle length (i.e., joint angle) the longer muscle (post stretch) should have a less passive tension (i.e., passive joint torque) than shorter muscle. Identically, longer muscle can tolerate more passive tension (higher peak torque). Stretching should induce a shift to the right in passive torque-angle relationship. Figure reprinted from Weppler and Magnusson (2010); Copyright 2010, with permission from Oxford University Press.*

As discussed in the previous sections, several acute and chronic stretching interventions have shown increases in maximal ROM in the absence of significant changes in mechanical properties of MTU (Weppler & Magnusson, 2010; Freitas *et al.*, 2017; Nordez *et al.*, 2017). These results underpin the conclusion that sensory mechanisms associated with stretch tolerance might be involved in stretching effects on maximal joint ROM. Specifically, when stretching increases muscle extensibility and tolerance to a greater tensile force in the absence of changes in muscle mechanical properties, the increases in ROM are suggested to occur at a sensory level: sensory-based theory (Figure 16). This sensory theory has been eloquently suggested by Weppler and Magnusson (2010). More recently, the findings of Freitas *et al.* (2017) support such a sensory mechanism, suggesting that the sensory perception of passive muscle tension may be crucial to alter muscle extensibility and to tolerate greater torque application (Weppler & Magnusson, 2010; Freitas *et al.*, 2017).

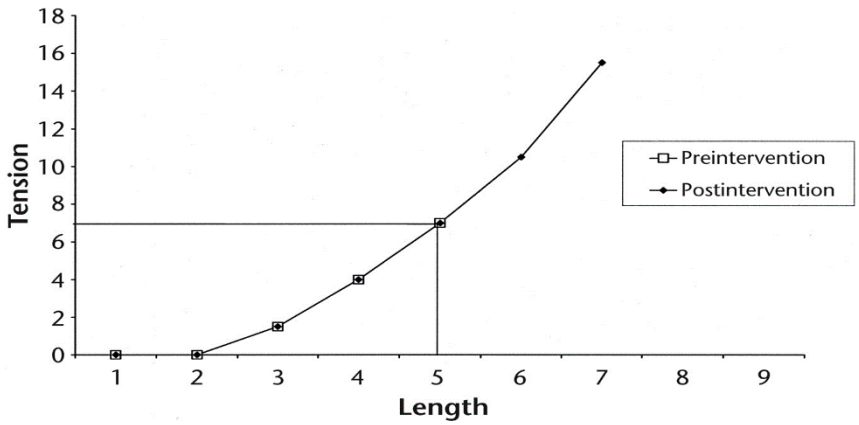


*Figure 16. Sensory theory to explain the maximal ROM adaptations after chronic stretching, assuming that torque-angle reflects the muscle passive length-tension. For the same length (i.e., joint angle) there is an absence of pre- to post-stretch intervention changes in tension (i.e., joint passive torque). In postintervention, muscle can tolerate a higher amount of tensile force and to extend to a higher length. No shift in torque-angle curve should occur. Figure reprinted from Weppler and Magnusson (2010); Copyright 2010, with permission from Oxford University Press.*

Interestingly, it has recently been observed that stretching also increases non-local maximal joint ROM, and thus the sensory theory effect might not be limited to the joint where the stretching took place. Specifically, Behm *et al.* (2016b) found an increase in ROM of lower body after a static/dynamic stretching of the upper limb (and vice-versa), suggesting that enhanced stretch tolerance was the key factor in the improved ROM rather than a mechanical or neural drive mechanism. Consistent with these findings, Chaouachi *et al.* (2017) observed that unilateral static/dynamic stretching provides increases in maximal ROM for both stretched and non-stretched limbs in the absence of changes in joint torque and myoelectric activity. The authors suggested that most of these changes can be explained by central mechanisms.

However, the stretching effects of these studies were of low magnitude with small effect sizes. More striking, though, after an acute single stretch intervention Mizuno *et al.* (2013b) observed that the decrease in joint passive torque has a shorter duration (i.e., time course effects) than improvements in maximal joint ROM. Therefore, although the results of the latter study clearly demonstrate an influence of joint mechanical properties on maximal joint ROM, a sensory mechanism cannot be totally excluded for the time course effects.

Overall, current evidence suggests that increases in muscle extensibility and maximal joint ROM observed after a single session and after chronic stretching programs (3 to 8 weeks) are mainly due to modified sensation. Furthermore, it was recently suggested that non-muscular

tissues may be more sensible to mechanical and sensory adaptations than skeletal muscle itself. The potential candidates are the connective tissues (i.e., fasciae) and peripheral nervous system, which would play an important role in the perception of stretch and in the maximal joint ROM, and little impact in global passive torque (Freitas *et al.*, 2017; Nordez *et al.*, 2017).

## 4. Non-muscular structures can limit maximal ROM

### 4.1. The important role of non-muscular structures

During joint movement other non-muscular tissues such as peripheral nerves and fasciae may experience large changes in their length and tension; however, the influence of these non-muscular tissues on the maximal joint ROM available at a joint remains largely unexplored. Although there is no direct evidence for the influence of these non-muscular tissues on maximal joint ROM, several findings from both observational and interventional studies have suggested that they may have an important role in the maximal joint ROM of a given joint. Indeed, both tissues are known to cross multiple joints and to have a dense sensory innervation (Dilley *et al.*, 2005; Tesarz *et al.*, 2011; Stecco & Hammer, 2015). Hence, they are highly sensible to mechanical stress or strain that may trigger pain that could affect maximal joint ROM when lengthened. Stretch interventions targeting individually these tissues could therefore change their mechanical behavior, and have a direct influence on maximal joint ROM. Although several studies have suggested both nerves and fascia as probable restrictors of maximal joint ROM, they have not been able to dissociate the effects of these structures due to their similarities in continuity network.

For instance, Wilke *et al.* (2017) recently found that an acute bout of hamstring and plantar flexors stretch is as effective as local neck stretching to improve cervical maximal ROM. Moreover, Le Sant *et al.* (2015) observed that during a single passive knee extension the peak passive stiffness of hamstring muscles (estimated at maximal knee extension angle by shear modulus) is higher when the hip is in a greater flexed position. However, assuming the mechanical theory, the stretch perception should be directly linked to the passive muscle tension. The results of the latter two studies (Le Sant *et al.*, 2015; Wilke *et al.*, 2017) provide evidence that other non-muscular factors may be involved in the maximal ROM mechanisms.

Furthermore, it has been observed that maximal knee extension ROM and hamstring extensibility are reduced in a slump position (lumbar and thoracic spine flexion; and hip flexion) when compared to a neutral position (Laessoe & Voigt, 2004; McHugh *et al.*, 2012; Andrade *et al.*, 2015). Interestingly, Mitchell *et al.* (2008) observed a remarkable decrease in the maximal ankle ROM in dorsiflexion when the hip was flexed from the neutral position to 90°. Because there is no muscle-tendon unit crossing both the hip and ankle joints, this result suggests that non-muscular structures may limit the maximal ankle ROM in dorsiflexion. The sciatic nerve that extends from the spine through branches to the foot and, like most other connective tissues, exhibits viscoelastic behavior seems to be the best candidate to explain this physiological limitation in ROM. However, due to its anatomical continuity, the influence of deep fascia of the lower limb cannot be excluded (Stecco & Hammer, 2015).

## 4.2. Relative contribution of fasciae

Both basic and applied research in fasciae have considerably increased over the ten years (Stecco *et al.*, 2011). However, the biomechanical characteristics of these tissues remain largely unexplored. Fasciae are continuous structures throughout the body. These densely banded connective tissues, where fibers have more than one dominant direction (Benetazzo et al., 2011), link the active components of the movement system creating an extensive network of myofascial bonds.

### *4.2.1. Structural organization and contributors to passive tensile force*

From the skin to the muscular level, three important fibrous connective layers can be considered in humans (apart from all the visceral fasciae): superficial fascia, deep fascia, and epimysial fascia (i.e., epimysium, also considered as a division of deep fascia) (Stecco *et al.*, 2011; Stecco & Hammer, 2015). The contribution of the epimysium to the passive tension of MTU was already described previously (for a review see section 2.5.1).

The superficial layer is a thin structure (66.6 μm ± 18.6 μm) formed by collagen fibers, loosely packed and mixed with abundant elastic fibers arranged irregularly. It is directly linked to the skin and provides support for subcutaneous structures. Retinacula link both superficial and deep fascia

in a three-dimensional network between the fat lobules (Stecco *et al.*, 2011; Stecco & Hammer, 2015). Deep fascia of the limbs, which is thicker than superficial fascia (~1 mm), is formed by an extensive network of fibrous bundles running in different directions (Gerlach & Lierse, 1990; Stecco & Hammer, 2015). Therefore, they are commonly classified as a dense irregular connective tissue, mainly formed by collagen type I (~18%) and fibroblasts (Benetazzo *et al.*, 2011; Stecco *et al.*, 2011). In some regions, it is possible to find other collagen fibers (types II and III), and their elastin content if relatively small (< 1%) (Stecco & Hammer, 2015). From a functional point of view, deep fasciae contribute to envelop a muscle (i.e., epimysial fasciae) or muscle groups (i.e., aponeurotic fasciae).

Together, the tri-dimensional arrangement of fascial layers and their histologic composition suggest that they can contribute to mechanical tension of musculo articular complex (Stecco & Hammer, 2015). Additionally, the spatial orientation of the collagen fiber bundles enables fasciae to provide passive resistive tension when tensile forces are applied in different directions (multiaxial loading).

### *4.2.2. Mechanical response of fasciae to loading*

The complex structural organization of fasciae is reflected in their mechanical properties. For instance, stress-strain curves up to failure point (Figure 17-A) have been described in mechanical tests performed in aponeurotic crural and plantar fascia fasciae of cadavers (Stecco *et al.*, 2014; Stecco & Hammer, 2015). These tests have demonstrated that fascial tissue behaves mechanically like other connective tissues, exhibiting a non-linear mechanical response to stretching. They have viscoelastic behavior in a physiological range of strain, illustrated by the presence of hysteresis (Figure 17-B).

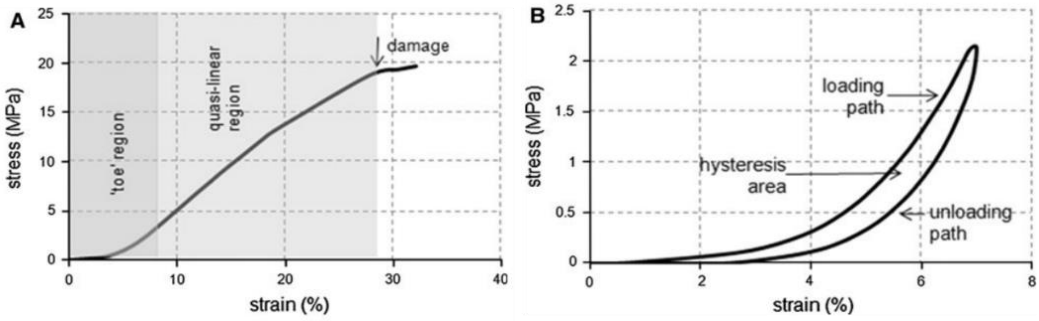


*Figure 17. Mechanical response of the crural fascia to stretching.* ***A.*** *Tensile test up to failure.* ***B.*** *Loading-unloading cycle with typical hysteresis loop. Figure reprinted from (Stecco et al., 2014); Copyright 2014, with permission from Elsevier.*

Anisotropic behavior has also been found. For instance, Hurschler et al. (1994) and Stecco et al. (2014) demonstrated that fasciae are stiffer when loaded in the longitudinal rather than in the transverse direction. In addition, relaxation tests at fixed strains have demonstrated that a decrease of stress about 32% may occur in within the first 120 s with a subsequent decline of 5% in the following 120 s. However, relaxation tests confirm that about 90 % of the stress decreases may happen in the first minute after the application of the strain, this being a response that is identical for both longitudinal and transversal axis (Hurschler *et al.*, 1994; Stecco *et al.*, 2014). Together, these results suggest that a 60 s of static stretching is enough to significantly induce substantial changes in localized fascia stiffness.

Interestingly, it has been observed that at low strain levels only the collagen fibers that are aligned in the direction of the applied force contribute to the passive resistance to stretching (Figure 18). As strain levels increase, more fiber bundles are progressively orientated along the loading direction (Stecco & Hammer, 2015).

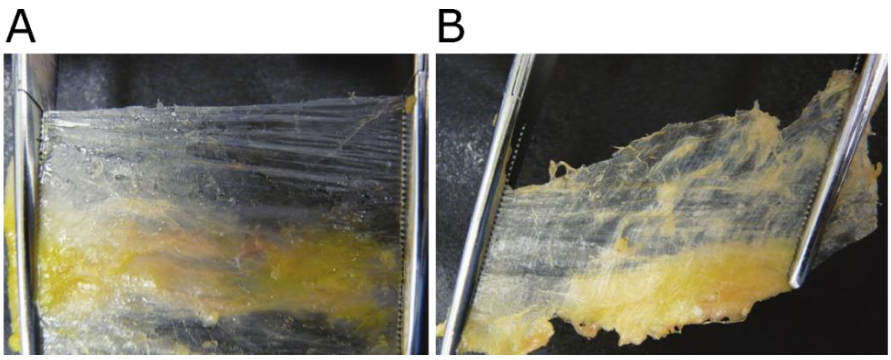


*Figure 18.* ***A.*** *Deep fascia stretched along longitudinal direction.* ***B.*** *Deep fascia stretched along oblique direction. Figure reprinted from Stecco and Hammer (2015); Copyright 2015, with permission from Elsevier.*

### *4.2.3. Can fasciae contribute to the limitation in maximal joint ROM?*

Thanks to their continuity anatomy throughout the body, fasciae may undergo deformation with limb's posture (multi-articular positioning), passive joint motion and muscle shortening (e.g., contraction). Thus, myofascial complex has been suggested to modulate the force transmission by muscles passing over the various joints and segments (Maas & Sandercock, 2010; Purslow, 2010; Yucesoy, 2010). For example, studies using ultrasound and resonance magnetic imaging have observed that changes in knee angle affected the fiber length of mono-articular muscles that do cross the knee joint such as soleus, deep flexor, tibialis anterior and peroneal muscle groups (Bojsen-Moller *et al.*, 2010; Huijing *et al.*, 2011; Yaman *et al.*, 2013). Thus, force transmission mechanisms could have occurred between these muscle and neighboring structures that cross the knee joint such as the *gastrocnemii* muscles. Together, these findings suggest that specific postures of lower limb can preferentially stretch fasciae.

More interestingly, a recent study showed that the displacement of the *gastrocnemius medialis* deep fascia is highly correlated with the pelvic motion (i.e., anteversion) (Cruz-Montecinos *et al.*, 2015), confirming *in-vivo* a myofascial connectivity between the trunk and lower leg as reported in anatomical studies (Stecco & Hammer, 2015).

Importantly, fasciae are richly innervated tissues and composed of abundant free nerve endings closely connected to the surrounding collagen fibers (Tanaka & Ito, 1977; Yahia *et al.*, 1992; Stecco *et al.*, 2007; Stecco & Hammer, 2015). This indicates that fasciae may be sensitive to stretching stimuli. Therefore, maximal joint ROM assessments involving significant lengthening of fasciae may influence the onset of stretch pain sensation and, thus, the stretch amplitude.

Overall, these findings suggest that fasciae have the potential to play a role in maximal joint ROM when mechanically loaded. However, they are thinner and much less organized (e.g., their course over, through, and around structures and joints of limbs) than other non-muscular structures such as peripheral nerves (Gray et al., 2012; Stecco & Hammer, 2015). Therefore, it is less probable that they undergo greater mechanical adaptations than peripheral nerves for an identical stretching stimulus. Additionally, the thickness of fasciae should be currently problematic for the assessment of their mechanical properties *in-vivo* using non-invasive technology, such as ultrasound shear wave elastography.

## 4.3. Relative contribution of peripheral nerves

As fasciae, peripheral nerves are continuous structures from spine to the extremities of limbs. For example, the thickness of the sciatic nerve may vary between 2 and 12 mm (Gray *et al.*, 2012; Kara *et al.*, 2012). Moreover, depending on the level of assessment, the width of the sciatic nerve can vary between 10 and 22 mm (Gray *et al.*, 2012; Kara *et al.*, 2012; Cho Sims *et al.*, 2016). Moreover, the scientific evidence underlying both the anatomy and biomechanics of peripheral nerves is considerably greater than those in fasciae. The following sections provide evidence for the complex structure of viscoelastic nerves, their possible relative contribution to the maximal joint ROM, how they respond mechanically to stretching, and adapt to lengthening stimuli.

### *4.3.1. Structural organization*

There are 31 pairs of spinal peripheral nerves distributed along 5 spinal cord segments (cervical, thoracic, lumbar, sacral and coccygeal), which are identified by its association with the vertebra where they leave the vertebral canal. At this level, ventral and dorsal nerve roots join together, when they exit the spinal cord through the intervertebral foramina, to form the peripheral nerves. Upon emerging from intervertebral foramina, most of the peripheral nerves branch out (plexuses that comprise trunks, divisions and cords) and, on occasion, recombine as they course through the body.

Peripheral nerves are an organized collection of axons with connective tissue (Figure 19). The axons in nerves are organized into fascicles, or bundles that collectively make up a nerve. At each organizational level, there is a layer of connective tissue provides compressive and tensile strength to the structure (Topp & Boyd, 2006). The endoneurium envelopes each axon. Within the endoneurium all axons are intimately associated with Schwann cells, which are surrounded by collagen type IV. Between the axons, there is a loose connective tissue collagen fibrils (type I and II) in longitudinal orientation (Thomas, 1963). Axons and Schwann cells provide minimal mechanical contributions for the structural integrity of axons. The perineurium is a dense connective tissue that encloses each fascicle. It can be formed by up to 15 layers of flat cells that are interspersed with layers collagen fibrils (type I, II and IV) and elastic fibers (Thomas, 1963; Gamble & Eames, 1964; Sunderland, 1990). These layers have circumferential, oblique, and

longitudinal orientations. The epineurium forms a tough, fibrous sheath around the entire structure of the nerve, which is formed by bundles of type I and type III collagen fibrils and elastic fibers (Stolinski, 1995). This layer is linked to paraneural fascial components of the connective tissue that surrounds the nerve (Millesi *et al.*, 1995).

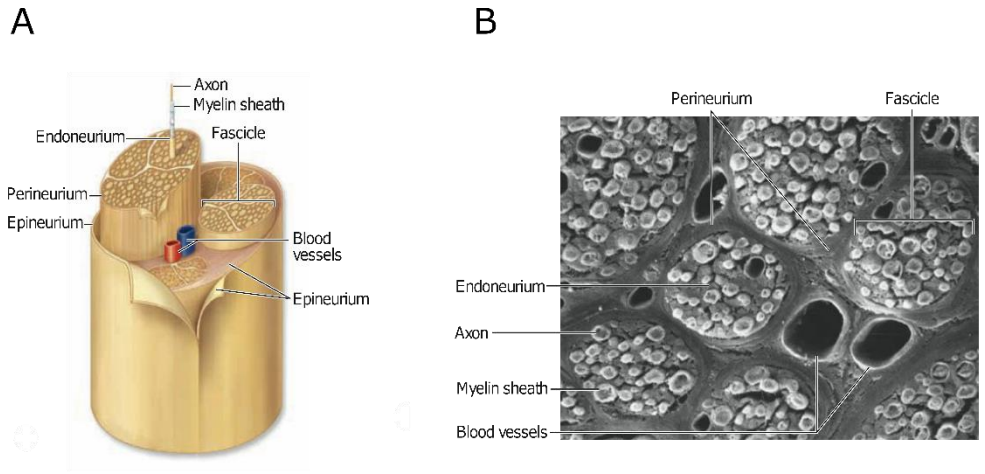


*Figure 19.* ***A.*** *The diagram demonstrates the relationship among the three connective tissue layers in large peripheral nerves;* ***B.*** *Scanning electron microscope of transverse sections of a large peripheral nerve showing several fascicles, each surrounded by perineurium and packed with endoneurium around the individual myelin sheaths. Each fascicle contains at least one capillary (×450). Figure reprinted from Mescher (2013); Copyright 2013, with permission from McGraw-Hill Education.*

### *4.3.2. Factors contributing to the passive stiffness of nerves*

The hierarchical structural organization and the mechanical properties of the connective tissue of the nerve contribute to the global resistance of nerve to stretch during articular motion. Like other soft tissues, the nerves display a viscoelastic behavior, such as stress-relaxation and creep, under loading (Millesi et al., 1995; Topp & Boyd, 2006). The passive tension produce by a stretched nerve is provided by a mixture of elastin and collagen content of the connective tissue in the nerve (mostly in perineurium and epineurium layers) and suggests that nerves have the capacity to undergo large deformations without functional loss (Topp & Boyd, 2006). Indeed, the perineurium has been considered the primary mechanical contributor to the overall passive tension and elasticity of a stretched nerve (Rydevik *et al.*, 1990; Sunderland, 1990), although the endoneurium provides trivial degree of tensile (Thomas, 1963; Sunderland, 1990). Moreover, the dense network of collagen and elastic fibers of the epineurium can dissipate tensile compressive

forces but should play a secondary role in the tensile offered by a stretched nerve (Haftek, 1970; Rydevik *et al.*, 1990).

Interestingly, the axons and fascicles within the nerve core are displaced in an undulating manner (Clarke & Bearn, 1972; Sunderland, 1990). When the overall nerve is stretched these undulations may allow substantial elongation within the nerve core (axons and fascicles) before developing significant tensile force. This structural arrangement may contribute to the behavior of nerve loading, and it has been considered as a protective mechanical mechanism of the relatively fragile axons. In addition, constant fluid pressure within endoneurium may also contribute significantly to viscoelastic of nerves (Phillips *et al.*, 2004). Furthermore, it has been suggested that the localized variation in collagen fibril diameter of endoneurium, perineurium and epineurium along the nerve (Mason & Phillips, 2011) is likely to contribute to the longitudinal heterogeneity of tensile properties in this nerve (Phillips *et al.*, 2004).

In addition, although nerves and nerve roots are displaced in series (continuous) with the nerve roots and spinal cord they are mechanically and structurally distinct from each other. As such, the mechanical properties of nerve roots may also affect the passive tensile response of nerves during stretching. Within the nerve roots, axons are enveloped within three layers or meninges: pia mater, arachnoid mater and dura mater, from deep to superficial, respectively. Interestingly, the epineurium of the nerve is continuous with the dura mater of nerve roots and spinal cord. The dura mater has been suggest to provide most of the passive tension of the nerve roots (Beel *et al.*, 1986) (Maikos *et al.*, 2008; Mazgajczyk *et al.*, 2012). Nerve roots were shown to be 83% less stiff than nerves (Beel *et al.*, 1986). The total amount of collagen in nerve roots is 3-7 times less than that of nerves and could explain such stiffness differences (Stodieck *et al.*, 1986).

In the nerve roots, the arrangement of axons and fascicles within the nerve core is more parallel than the one observed in nerves (Sunderland, 1990). However, a wave-like pattern in the fibers of the dura along the nerve length has also been observed. Several studies performed in cadavers or *in-vivo* (intraoperatively or using magnetic resonance image) have shown that when the entire nerve system is stretched, nerve roots may undergo 1-4 % strain (Smith *et al.*, 1993; Gilbert *et al.*, 2007) and experience significant longitudinal displacements, causally, by 1-8 mm (Smith *et al.*, 1993; Kobayashi *et al.*, 2003; Gilbert *et al.*, 2007; Rade *et al.*, 2014). The armament of the plexuses may also contribute to the transmission of forces between principal nerve branches and the nerve

roots (Breig & Troup, 1979). Together, this suggests that the mechanical behavior of nerve and nerve roots differs when loaded along their long-axis, and that the tensile properties of the stretched peripheral nerve are influence by the mechanical behavior of nerve roots.

### *4.3.3. Mechanical response of peripheral nerves to loading*

From a series of biomechanical studies performed in cadavers, Breig and Braxton (1978) showed that movement of the cervical spine influences movement at the lumbar dural sheath ,and vice versa. Because of this connectivity, peripheral nerves have also been related to limitations in maximal joint when they are mechanically loaded (Nordez *et al.*, 2017). Their specific structural organization enables them to tolerate and adapt to stresses induced by joints' motion (Topp & Boyd, 2006; Shah, 2017). As such, the nerve can be stretched independently of its surrounding muscular and non-muscular tissues (Coppieters *et al.*, 2006). The mechanical loading of these continuum structures can potentially limit the maximal ROM in certain joint and/or limb configurations. Although direct evidence still is lacking, strong suggestive findings have contributed to expanding this theory (Nordez *et al.*, 2017).

Mechanical properties of nerves have been extensively examined *in-vitro* and *in-situ* using animal specimens (Rydevik *et al.*, 1990; Kwan *et al.*, 1992; Millesi *et al.*, 1995; Topp & Boyd, 2006). Nerve responses to loading depend on the degree of deformation. For example, a rate-dependent response has been observed, demonstrating that nerve stiffness increases by increasing strain rates, which reflects the viscoelastic nature of this tissue (Tillett *et al.*, 2004; Kassab & Sacks, 2016). Typical load-elongation and stress-strain curves have been used to estimate nerve stiffness from their slope (Figure 20) (Kwan *et al.*, 1992). A steeper slope indicates a stiffer nerve and less compliant nerve to elongation. In contrast, a shallow slope represents a less stiff, but more compliant nerve (Topp & Boyd, 2006).

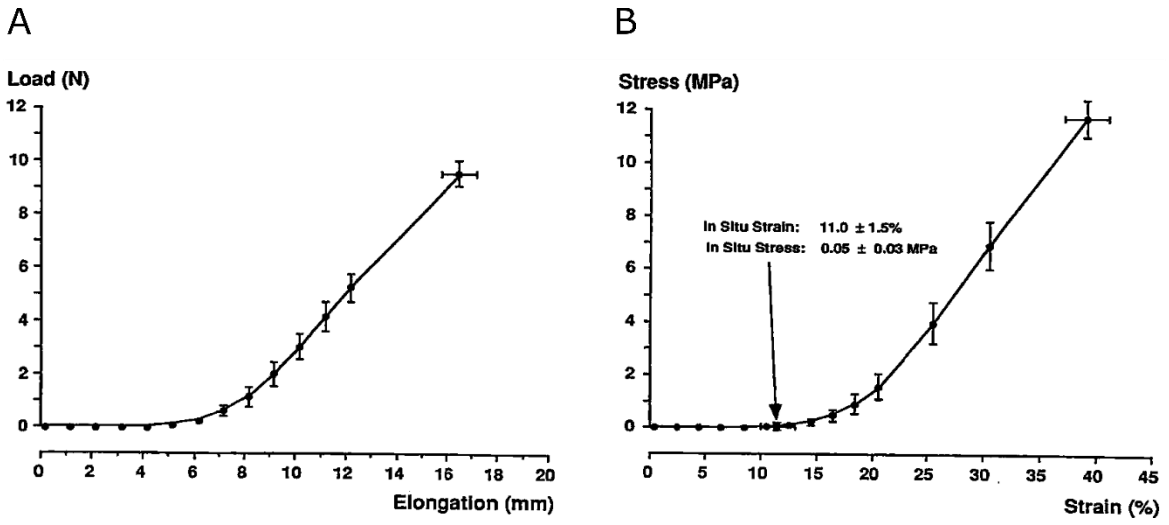


*Figure 20.* ***A.*** *Typical load-elongation curve for rabbit tibial nerve. The nerve can be stretched a large amount before substantial tension has developed.* ***B.*** *Non-linear tress-strain curve for rabbit tibial nerve. In situ, the nerve is under large strains, but minimal stresses. Figure reprinted from Kwan et al. (1992); Copyright 1992, with permission from Taylor & Francis, Inc.*

As skeletal muscles, nerves experience increases in tension when lengthened beyond their slack length (Millesi *et al.*, 1995; Topp & Boyd, 2006). In nerves, though, the slack range may also be affected by the undulating course of axons (i.e. bands of Fontana) and nerve fascicular bundles (Clarke & Bearn, 1972; Haninec, 1986). Hence, slack length point of nerves should only occur once the undulation becomes straightened out or disappears. This special undulating arrangement of both collagen fibers of the epineurium and endoneurium may induce additional slack to accommodate initial elongation forces.

### *4.3.4. Adaptations of nerves to stretching*

Peripheral nerves are remarkable tissues characterized not only by a complex electrophysiological network linked by axons, but also by their notable mechanical properties. Paradoxically, though, they are not often considered as mechanical structures as most of other tissues (e.g., muscle, bone, ligaments or cartilage), which may also explain the knowledge gap underlying the biomechanical adaptations of nerves to both acute and chronic stretching *in-vivo*.

Several animal experiments have tested the effects of stretching on viscoelastic nerve structure (Rydevik et al., 1990; Driscoll et al., 2002) and, specially, to identify the mechanical threshold to which nerves can be stretched (i.e., magnitude of stretch) without altering their conduction

properties and intraneural blood flow supply (Wall et al., 1991; Kwan et al., 1992; Ma et al., 2013). Creep and hysteresis phenomena have been observed animal nerves (Kendall et al., 1979).

The mechanical response of the nervous system to loading should be modulated by the conditions of loading, such as the magnitude and rate of lengthening (Bueno & Shah, 2008). For example, Kwan et al. (1992) demonstrated that when a nerve is elongated to 9-12% above their resting lengths a stress relaxation occurs, mostly in the first 20 min. The authors showed notably that the degree of relaxation is affected by the percentage amount of elongation (Figure 21).

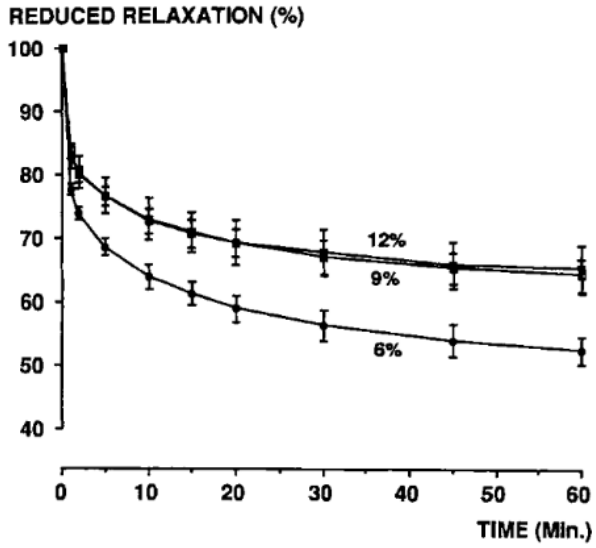


*Figure 21. Relaxation of peripheral nerve (rabbit) when stretched to fixed strain levels of 6%, 9%, and 12% above the in situ length (slack length), during 60 min. Figure reprinted from Kwan et al. (1992); Copyright 1992, with permission from Taylor & Francis, Inc.*

Interestingly, Millesi *et al.* (1995) observed that after a 60 min nerve stress relaxation at 5% strain stretching, the stress required to achieve the same amount of elongation on the subsequent stretching was considerably less. However, when a 10 min. recovery interval is introduced between the first and the second stretches, the load necessary to achieve the target elongation is the same (Millesi *et al.*, 1995). Interestingly, though, nerve strains during limb movement should not be equally distributed along their length, as it is increased at the articulation level (Phillips *et al.*, 2004). Collectively, these findings suggest that nerves can respond and adapt transiently to short lengthening mechanical stresses, and that the level of such loading dictates the adaptive mechanical response of neural tissue. However, it is unknown whether such adaptations to stretch

are homogenous along the nerve course, and if they could induce changes in joints' motion (i.e., maximal ROM).

In humans, the study of nerve mechanical properties has remained more elusive (Nordez *et al.*, 2017; Shah, 2017). During the last decade, solid attempts have been made to explore the biomechanics of nerves during human movement. Primary evidence, though, refers to human cadaveric research, in which measurements of tissue strains have been useful to study the nerves response to loading as joints/limbs are moved in multiple postures (Coppieters *et al.*, 2006; Alshami *et al.*, 2008; Coppieters & Butler, 2008; Boyd *et al.*, 2013). Notably, these works have demonstrated that nerves are continuous structures that can be manipulated by distant joints in the absence of strain changes in surrounding musculoskeletal tissues (Coppieters *et al.*, 2006; Topp & Boyd, 2006). Ultrasonography was further introduced to study *in-vivo* both structural properties (e.g., cross sectional area) and nerve displacements as a response to the articular movement and posture (Boyd *et al.*, 2012; Ellis *et al.*, 2012; Ridehalgh *et al.*, 2014; Coppieters *et al.*, 2015). Collectively, these findings have shown that nerves are exposed to significant mechanical stresses during movement as they elongate and slide relative to adjacent tissues (Topp & Boyd, 2006; Ellis *et al.*, 2012; Silva *et al.*, 2014). However, nerve displacement measurements cannot be used to infer on changes in stiffness or force, because the nerves may experience changes in their length within a slack length range while their stiffness or tension does not vary. Therefore, quantification of nerve excursion/sliding may have little impact on explaining the limitations in joint ROM associated to nerve elongation.

The influence of positions of different postures on nerve stiffness and tensile load also remains largely unexamined (Nordez *et al.*, 2017). The application of ultrasound shear wave elastography may provide a tool for examining nerve biomechanics in healthy individuals and patients, exploring a new field of research in human biomechanics. Elastography measurements to study mechanical properties of nerves have emerged during the last three years, which is concomitant with the period of this doctoral research. For instance, the study 2 of this thesis (Andrade *et al.*, 2016b) was the first study using this technique to characterize biomechanical properties of nerves as a response articular motion.

More recently, Greening and Dilley (2017) observed that posture such as straight leg raising at end ROM increases nerve stiffness (estimated by shear modulus), confirming previous findings of

cadaveric and ultrasound based research (Coppieters *et al.*, 2006). In addition, although changes in nerve tissue stiffness and structural properties have also been recently reported in context of chronic musculoskeletal (Kantarci *et al.*, 2014) and neurological (Dikici *et al.*, 2016) pathologies, such measurements taken in resting positions should not reflect dynamic adaptations of neural tissue to movement or imposed stress.

Despite this recent interest, little regard has been paid to mechanical adaptations of nerves *in-vivo* following a mechanical stimuli within a physiological pain range (Nordez *et al.*, 2017; Shah, 2017). Osteogenic lengthening procedures, such as distraction osteogenesis (e.g., Ilizarov procedures), have shown that nerves and skeletal muscle tissues have the same adaptation and regeneration potential just like the osteocyte. For example, morphological changes (i.e., internode length and deposition of endoneurial collagen) have been observed in a rodent model at slow elongation rates per day, from 14 to 72 days, which suggests great ability for adaptation of a gradually elongated nerve (Shibukawa & Shirai, 2001; Hara *et al.*, 2003; Abe *et al.*, 2004). If present, such tissue modifications may represent strong clinical importance, in particular to the understanding and the management of nerve tissue in the context of entrapment neuropathy or chronic neuropathy, where nerve architecture and stiffness are known to be affected, as well as neural and joint's function.

### *4.3.5. Can peripheral nerves contribute to the limitation in maximal joint ROM?*

Nerves cross multiple joints and course over, through and around a variety of anatomical structures that move during human motion. Nerves must experience large changes in their length and tension (i.e., stretch, twist, and bend) to accommodate normal movements, such as joint motion and muscle contraction, without damaging the axons within (Topp & Boyd, 2006).

For instance, Halbertsma *et al.* (1996) found an increase in hip ROM in the absence of mechanical changes in hamstring MTUs after one session of static stretch. Interestingly, the straight leg raising test used for the intervention is also known to induce stretching in the sciatic nerve (i.e., increased strain in tibial branch) without affecting surrounding musculoskeletal structures (Coppieters *et al.*, 2006). Hence, improvements in maximal ROM could have been proposed to explain increases on ROM (Nordez *et al.*, 2017). Additionally, Gajdosik *et al.* (1985) and Boyd *et al.* (2009) found a ~10° significant decrease in maximal flexion ROM when

performing the same straight leg raising test with the ankle dorsiflexion compared with a testing with ankle in plantar flexion (knee fully-extended).

Furthermore, the majority of the studies that point to a possible role of non-muscular structures on maximal joint ROM have used clinical tests, such as slump and straight leg raising as stretching maneuvers (Gajdosik *et al.*, 1985; Laessoe & Voigt, 2004; Mitchell *et al.*, 2008; Boyd *et al.*, 2009; McHugh *et al.*, 2012; Andrade *et al.*, 2015). These clinical tests are known to put mechanical stress on the peripheral nerves, and are used to test the mechanosensitivity of neural tissues (Butler, 1991; Shacklock, 2005; Coppieters *et al.*, 2006).

Concomitant with these observations, a recent systematic review showed that neurodynamic techniques can induce significant improvements in maximal joint ROM in both healthy and low back pain populations (Figure 22) (Neto *et al.*, 2017). However, neurodynamic techniques include combinations of joint movements that promote either neural tensioning (displacement of the nerve endings in opposite directions) or sliding (displacement of nerve endings in the same direction) (Coppieters & Butler, 2008). As other structures such as MTU may be loaded during the articular motion imposed to manipulate the nerves, the effects of neurodynamic techniques on maximal ROM should be analyzed with caution. In summary, there is a knowledge gap in the relationship between the mechanical loading of nerves and its effects on human maximal ROM of a given joint.

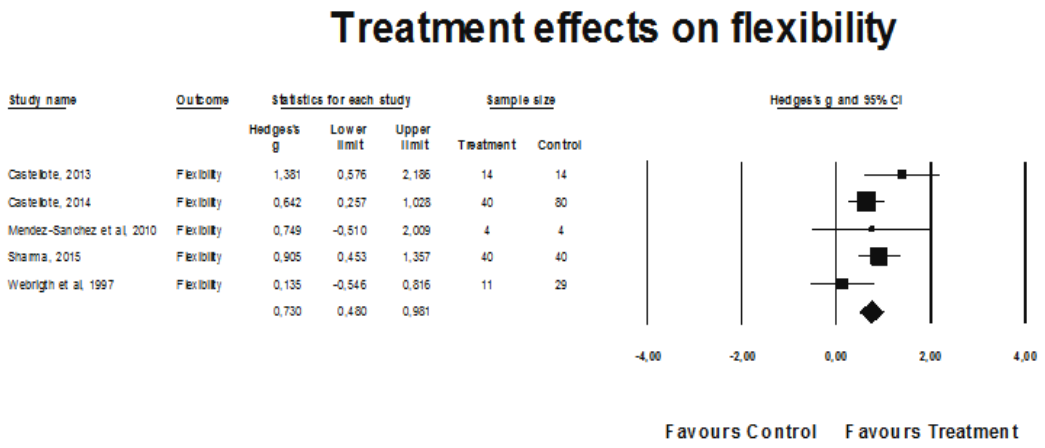


*Figure 22. Forest plot of the effects of neurodynamic techniques on lower limb flexibility. Figure reprinted fromNeto et al. (2017); Copyright 2017, with permission from Elsevier.*

In addition, peripheral nerves sheaths are innervated by intrinsic nerves such as the nervi nervorum, which are composed of unmyelinated or thin myelinated fibers consisting of a thin plexus layer of free nervous endings in all layers, and some encapsulated Pacinian corpuscles (Figure 23) (Hromada, 1963; Bove & Light, 1995b; Bove & Light, 1997; Bove, 2008). Nervi nervorum is very sensitive to excessive or very localized stretching in longitunigal axis of the nerve, but it should not be active during nerve stretching within short movement ranges (Bove & Light, 1995a; Bove & Light, 1997). Thus, during maximal ROM assessements in which peripheral nerves are maximally stretched (within a physiological range) before the MTUs, the mechanical lengthening of nerve sheats should trigger the activation of nervi nervorum and determine the stretch amplitude.

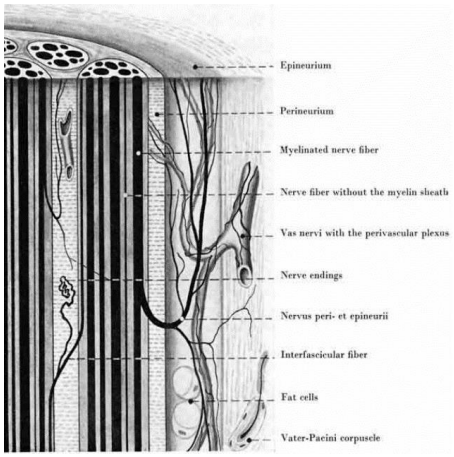


*Figure 23. Schematic sources and terminations of nervi nervorum (i.e. vas nervi) within the sheaths of a nerve. Figure reprinted from Hromada (1963); Copyright 1963, with permission from Karger.*

### *4.3.6. Sciatic nerve*

The mechanical properties of the sciatic nerve and its possible role in the limitation of maximal dorsiflexion ROM were studied in this thesis. Therefore, the following sections will briefly describe the specific anatomy of sciatic nerve and how it can be stretched by manipulating several joints including the lumbar spine, hip, knee and ankle joints.

#### *4.3.6.1. Anatomy*

The sciatic nerve (Figure 24) is the longest and thickest nerve of the human body. Although it bifurcates in tibial and common fibular nerves when it reaches the apex of the popliteal fossa (may be at different levels in popliteal fossa or even more proximally), the sciatic nerve is considered a continuous anatomical structure (Saleh *et al.*, 2009; Netter, 2010; Gray *et al.*, 2012).

It is formed in lumbosacral plexus by the joining of anterior branches of L4-S3 spinal nerves, leaving the pelvis and entering on the gluteal region via greater sciatic foramen (Gray *et al.*, 2012). After emerging inferiorly to the piriformis muscle, it progresses in an inferolateral direction between the greater trochanter and ischial tuberosity. Then, it courses through the posterior thigh by passing deep into the long head of the *biceps femoris*. At this level, it is surrounded by a single epineural sheath before dividing into its two main branches. The common fibular nerve progresses to the leg in an inferior and lateral direction, and terminates by dividing into the superficial fibular and deep fibular nerves. These nerves will further cross the ankle joint by passing anterior to the distal tibia. In contrast, the tibial nerve (contribute with branches to sural nerve), runs down the leg posterior to the tibia. At the ankle joint, the tibial nerve passes posteriorly and inferiorly to the medial malleolus (i.e., tarsal tunnel), and then terminates by diving into sensory branches.

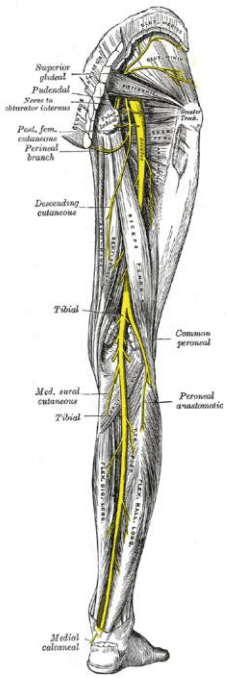


*Figure 24. Sciatic nerve tract (yellow), and its main branches below the knee region (tibial and fibular/peroneal). Posterior view of the lower limb. Figure reprinted from Gray et al. (2012); Copyright 2012 with permission from Octopus Publishing Group.*

#### *4.3.6.2. Biomechanical response to lower limb postures*

Due to the multi-joint topographic arrangement, the individual positioning of each joint in which they course through (lumbar spine, hip, knee and ankle) can notably affect the mechanical loading of the whole sciatic nerve tract. Thus, mechanical elongation at one site in the sciatic nerve can produce a cascade of related events along the system (e.g., changes in stiffness), which can occur distant from the moving joint (i.e., neighboring joints).

For instance, straight leg raising test (i.e., Lasègue test) is often used in clinical practice to test the movement and mechanical sensitivity of the lumbosacral neural structures and their distal extensions, including not only lumbosacral nerve roots and plexuses but also sciatic nerve tract (Butler, 1991; Shacklock, 2005). The test consists of stretching the entire sciatic nerve course by flexing the hip joint with a straight knee. Additional tension in the nerve and related structures such as the nerve root and trunks is thought to be provided by ankle dorsiflexion, the so called sensitizing maneuvers (Goddard & Reid, 1965; Breig & Troup, 1979; Troup, 1981; Coppieters *et al.*, 2006; Boyd *et al.*, 2013).

For instance, maximal hip flexion ROM during straight leg raising test is limited by these sensitizing maneuvers (e.g., ankle dorsiflexion) that may further elongate the sciatic nerve system (Breig & Braxton, 1978; Gajdosik *et al.*, 1985; Coppieters *et al.*, 2006). In other words, ankle dorsiflexion may significantly reduce the maximal straight leg raising ROM. Additional tension on the entire sciatic nerve course has been identified as a potential factor limiting hip joint ROM (Gajdosik *et al.*, 1985). Concomitant with such a theory, cadaver studies have posteriorly demonstrated that straight leg raising induces significant strain in the sciatic nerve and may increase with further ankle dorsiflexion (Coppieters *et al.*, 2006; Alshami *et al.*, 2008; Boyd *et al.*, 2013).

Interestingly, although the greatest strain and nerve gliding may occur nearest the moving joint, the strain should also be transmitted along the nerve course well beyond the neighboring joints (Coppieters *et al.*, 2006). Moreover, ultrasound imaging studies have shown that knee flexion may fold the sciatic nerve system and significantly reduce the ability of nerve to glide/slide during proximal or distal joint motions (Coppieters et al., 2015). Conversely, knee extension increases the length of the sciatic nerve bed by up to 60 mm (Shacklock, 2005). More interestingly, recent

findings suggest that there is no proximal-to-distal and distal-to-proximal changes in sciatic nerve strain and net nerve excursion in the end position of straight leg raising tests (Boyd *et al.*, 2013).

Together, these findings suggest that sciatic nerve tract can be manipulated by combining specific postures of the lower limb. Thus, hip flexion with the knee fully-extended should stretch the sciatic nerve tract. The addition of ankle dorsiflexion to this "long-sitting" posture may maximally stretch the sciatic nerve.

## 5. Summary & limitations within the literature

Maximal joint ROM is an important functional parameter to estimate the passive muscle-tendon unit extensibility or the ability of muscle-tendon unit to lengthen, which is often used in clinical practice/investigations and sports. The tension developed within muscle-tendon units being stretched was considered as the principal factor limiting maximal ROM (Gajdosik, 2001). However, there is a growing body of research that suggests that acute and chronic stretching can induce increases in maximal joint ROM in the absence of mechanical adaptations in MTU (Freitas *et al.*, 2017).

Therefore, sensory central adaptations to stretch interventions have been suggested to explain improvements in ROM, rather than mechanical theories (Weppler & Magnusson, 2010). Although methodological limitations such as intensity and time under stretching can explain the lack of mechanical adaptations in MTU following a stretch regimen, there are several studies that provide indirect evidence for a mechanical influence of non-muscular anatomical structures on maximal ROM limitation (Nordez *et al.*, 2017). This hypothesis is likely to be valid in certain configurations where such structures are thought to be highly stretched. Specifically, fasciae and peripheral nerves have been identified as the best candidates, because they cross multiple articulations (Nordez *et al.*, 2017). Like skeletal muscles, nerves have a viscoelastic behavior and may adapt mechanically to stretching (Millesi *et al.*, 1995; Topp & Boyd, 2006). However, in the literature, they are not often considered as a mechanical structure, nor as having a functional role in the limitation of maximal joint ROM. This may also explain the knowledge gap underlying the biomechanical adaptations of nerves to a single and long-term stretching stimuli (Nordez *et al.*, 2017; Shah, 2017).

## 6. Aims and hypothesis of the thesis

This thesis aimed to extend the knowledge of the mechanical role of muscular and non-muscular tissues underpinning the maximal joint ROM at a given joint. To this end, individual adaptations to acute and chronic stretching stimuli were examined to reveal the specific mechanical contribution of each structure.

As one of the most studied joints in the field of flexibility in both healthy and clinical populations, and having an important functional role (e.g., gait), the ankle joint was chosen for our testing procedures. In all studies, sciatic nerve (non-muscular structure) and *triceps surae* muscles (muscular structures) were used to determine how they mechanically respond to individual passive loading, and whether they regulate the maximal dorsiflexion ROM of the ankle. The thesis is presented in a paper base style. Four studies were conducted with specific goals.

A previous study showed notable restrictions in ankle dorsiflexion ROM when flexing the hip joint to 90° from a neutral position (Mitchell *et al.*, 2008). Because there is no MTU that links both hip and ankle joints, such a result suggests that the loading of non-muscular structures could explain these ROM differences. However, no research has examined both the joint and muscle mechanical responses to stretch during such ankle ROM assessments, where the hip joint position is significantly modified. As such, the first goal of this thesis was to examine whether or not the joint and muscle mechanical responses explains changes in ankle dorsiflexion ROM when the angle of hip and cervical joints are modified (study 1). To this end, we simultaneously measured the joint ankle torque and *gastrocnemius medialis* shear modulus (an index of stiffness) during passive ankle dorsiflexion movement through a combination of hip and head position tests.

The second goal was to take advantage of ultrasound shear wave elastography to assess non-invasively the reliability of shear wave velocity measurements (an index of stiffness) during ankle motion (study 2). Additionally, it aimed to characterize the effect of passive dorsiflexion on sciatic nerve stiffness when the knee was in 90° flexion (knee 90º) or fully-extended; and the effect of five repeated dorsiflexions on the nerve stiffness. Due to its particular mechanical relationship with knee and ankle joints, we hypothesized that the sciatic nerve stiffness would increase during both ankle dorsiflexion and knee extension.

The third goal was to investigate the role of the sciatic nerve stiffness in the maximal ankle ROM in dorsiflexion (study 3). An experimental protocol was conducted in humans to determine whether or not a total of 6 min. static stretch that aimed to load the sciatic nerve without stretching the plantar flexors is effective to: i) alter nerve stiffness; and ii) increase the ankle's maximal ROM. To this end, the sciatic shear wave velocity (an index of stiffness) was measured, before and after the 6-min. static stretch, while the nerve was progressively stretched during ankle dorsiflexion.

Lastly, the fourth goal was to appreciate the passive mechanical adaptations of muscular and neural tissues after long-term stretching protocols designed to target each structure separately; and to reveal the functional role of each tissue (muscular and non-muscular) in the maximal ROM (study 4). For this purpose, the passive stiffness (estimated from shear wave velocity) of sciatic nerve and *triceps surae* muscles were measured using ultrasound shear wave elastography before and after two 12-weeks stretch training protocols. We hypothesized that long-term chronic stretching protocols induce specific mechanical adaptations in nerves and muscles that contribute to improve ankle flexibility.

*Chapter Three*

# METHODS

## 1. Background

The present section aims to summarize the methods and instruments used in this doctoral research that are common to all studies. Two main testing positions based on the hip joint position, HIP-neutral (Figure 25-A) or HIP-flexed (Figure 25-B), were used to preferentially load either the plantar flexors or the sciatic nerve, respectively. These positions were used to perform maximal dorsiflexion ROM assessments in studies 1, 3, and 4, and were used in both acute and chronic stretch interventions (study 3 and study 4, respectively). In study 2, participants were tested for only one position (HIP-neutral).

This chapter presents scientific evidence for the use of these positions in muscular and non-muscular maximal ROM assessments. In addition, this chapter provides the participant's characterization, equipment and main outcomes and the data processing procedures. Ultrasound shear wave elastography and isokinetic dynamometry (to assess ankle torque and joint angle) were the main instruments used to address our research questions. Although specific characteristics of these methods were already described in the literature review, this chapter summarizes the procedures used for both data collection and data processing.

## 2. Participants

A total of 94 healthy volunteers were involved in the four studies (Table 1). Participants were recruited locally from the Faculty of Sports Sciences and in the School of Physiotherapy (IFM3R, Nantes). Healthy individuals were selected, as our intention was to test the influence of normal nerve and *triceps surae* biomechanics on maximal dorsiflexion ROM, and thus eliminate potentially confounding variables associated with dysfunction. In all studies, participants had to meet inclusion criteria. Volunteers with ongoing musculoskeletal lower limb and spinal injuries, neuromuscular diseases, and orthopaedic-related problems were excluded. Additionally, all volunteers were tested for slump and/or straight leg raising to assess the mechanosensitivity of the nervous system. These tests had to be negative for the participant to be included in the study. Volunteers were systematically instructed not to practice vigorous exercise 24 h to 48 h before the evaluation session so that no confounding changes in muscle stiffness were introduced

(Lacourpaille et al., 2017). In each study, participants were informed about methods used before providing their written informed consent. The Institutional Ethics Committee (Tours Ouest I, reference: no. CCP MIP-08) approved the four studies, and all procedures conformed to the Declaration of Helsinki with its recent modification of Fortaleza (2013).

*Table 1. Averaged demographic characterization of participants and number of evaluation moments, per study*

| Study | Group | n | Sex | Age (years) | Height (cm) | Weight (kg) | Number of evaluations |
|---|---|---|---|---|---|---|---|
| **1** | All | 9 | 9♂ | 25 ± 3 | 181 ± 6 | 73 ± 8 | 1 |
| **2** | All | 10 | 10♂ | 25 ± 3 | 182 ± 7 | 76 ± 8 | 1 |
| **3** | Control and Stretch | 15 | 13♂, 2♀ | 22 ± 3 | 175 ± 7 | 66 ± 7 | 2 |
| **4** | Control | 18 | 9♂, 9♀ | 21 ± 2 | 172 ± 9 | 62 ± 9 | 2 |
| | Stretch 1 | 21 | 12♂, 9♀ | 20 ± 1 | 174.7 ± 9 | 66 ± 9 | 2 |
| | Stretch 2 | 21 | 10♂, 11♀ | 21 ± 2 | 171 ± 9 | 66 ± 9 | 2 |

*Legend: ♀, female; ♂, mal.*

## 3. Dynamometry

In all the studies, an isokinetic dynamometer was used to perform passive ankle rotations and to measure the ankle angle and passive torque. A Biodex system (System 3; Biodex Medical Systems, NY, USA) was used in studies 1, 2, and 4, while study 3 was conducted using a Con-Trex (Con-Trex MJ; CMV AG, Dubendorf, Switzerland). Specifically, the lateral malleolus was used to estimate the center of rotation of the ankle and was aligned with the axis of the ergometer. The neutral position of the ankle (0º) was defined as an angle of 90º between the footplate and the shank. The foot was firmly strapped such that the potential heel displacement from the dynamometer platform was minimized during the ankle rotations.

All measurements were performed on the right ankle joint. Angle and torque data were collected at 1 kHz (studies 1 and 2) or 100 Hz (studies 3 and 4) by analogue/digital converters. The following acquisition systems were used in the studies: for studies 1 and 4 the MP35 (Biopac Systems Inc., Goleta, USA) was used; and studies 2 and 3 used the PowerLab (ADInstruments, Colorado Springs, CO, USA).

## 3.1. Maximal dorsiflexion ROM assessments

Based on anatomy and passive mechanical properties of both the plantar flexors and the sciatic nerve tract, two testing positions were designed to passively assess the maximal dorsiflexion ROM: HIP-neutral (Figure 25-A) and HIP-flexed (Figure 25-B). Each position aimed to preferentially stretch either muscular – plantar flexors (HIP-neutral) – or non-muscular structures – sciatic nerve (HIP-flexed). A standard goniometer (MSD, Londerzeel, Belgium) was used to set the hip position.

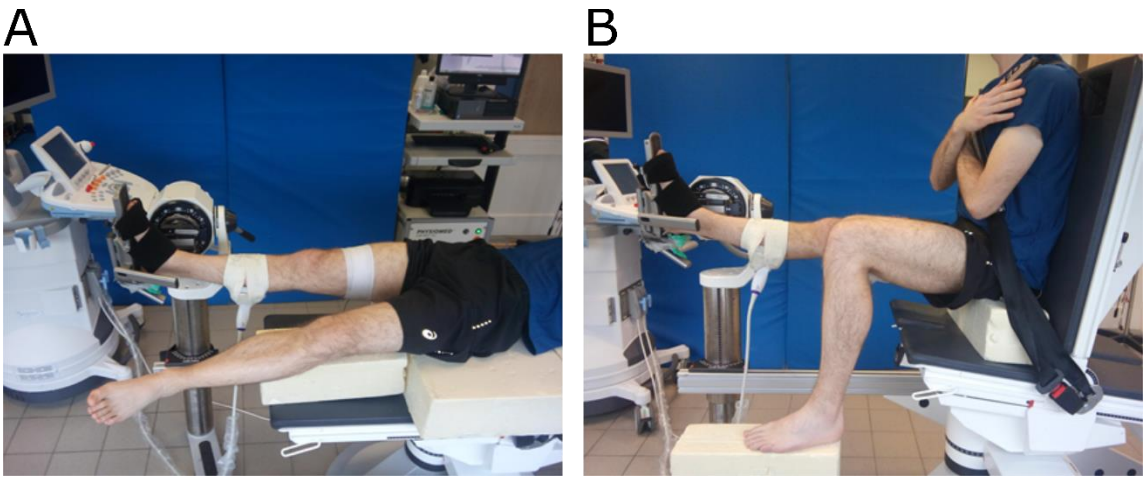


*Figure 25. Testing positions used to assess the maximal dorsiflexion ROM in the studies 1, 3, and 4.* ***A.*** *HIP-neutral position.* ***B.*** *HIP-flexed position. The pictures refer to study 3, which was performed by using a Con-Trex (Con-Trex MJ; CMV AG, Dubendorf, Switzerland). Studies 1, 2, and 4 were performed using a Biodex system (System 3; Biodex Medical Systems, NY, USA).*

These postures were used in studies 1, 3, and 4 in a random order. For study 2, only the HIP-neutral position was used. For the HIP-neutral testing, participants laid prone (study 4) or supine (studies 1 and 3) on the dynamometer system. The contralateral limb remained straight in a relaxed position. The knee was fully extended and strapped securely in this position such that no knee motion occurred during ankle rotations. For the HIP-flexed testing, participants were seated with the hip flexed at 90º and the knee fully extended. The contralateral limb was flexed to 90° (hip and knee) and remained relaxed. The chest and waist were both strapped to minimize trunk motion during ankle rotations. In study 2, volunteers were only tested in the HIP-neutral position (prone).

Before the maximal dorsiflexion ROM assessments, five ankle rotations were performed at 5°/s from 40° in plantar flexion to 15° in dorsiflexion in the HIP-neutral position to account for

the conditioning effects (Nordez et al., 2008b). Then, for the maximal joint ROM assessments, participants were asked to completely relax with their eyes closed while the ankle was passively rotated at 2°/s from 40° plantar flexion toward maximal dorsiflexion. Two trials separated by 1 min rest were performed for each testing position. Participants were instructed to reach their maximum stretch limit, which was defined as the onset of stretch pain in the posterior region of the leg or thigh. When they reached that point, they pressed a button that immediately released the footplate, hence ending the stretch on the soft tissue structures. The highest ankle ROM in dorsiflexion attained during these trials was considered the maximal joint ROM for statistical analysis procedures (Blazevich et al., 2014).

The standard error of measurement (SEM) of passive maximal dorsiflexion ROM assessments was calculated in study 4 to assess the intra-day reliability. Maximal dorsiflexion ROM assessments from both evaluation moments were used (n=118). SEM scores were 1.02° and 1.11° for HIP-neutral and HIP-flexed, respectively.

## 4. Ultrasound shear wave elastography

Ultrasound shear wave elastography was the principal assessment technique used in this doctoral research, with the tissue stiffness as the main outcome. Elastography was used to quantify the shear modulus of individual structures, and thus dynamically assess the passive stiffness of *triceps surae*, including the *gastrocnemius medialis* (studies 1, 3, and 4), *gastrocnemius lateralis* (study 4), and soleus (study 4). In addition, the long head of the *biceps femoris* (study 3), sciatic (studies 2, 3 and 4), and tibial (study 4) nerves were also evaluated. For all the studies, measurements were performed during passive ankle rotations from 40° plantar flexion to maximal dorsiflexion ROM at 2°/s. These measurements allowed us to examine: the muscle stiffness behavior to passive stretching (study 1); the reliability of sciatic nerve stiffness measurements during joint motion (study 2); and the acute (study 3) and chronic (study 4) effects of stretching protocols on both sciatic nerve and *triceps surae* stiffness. In all studies' elastography measurements were performed in the HIP-neutral position. Additionally, in study 1, *gastrocnemius medialis* measurements were performed in the HIP-flexed position.

An Aixplorer ultrasound scanner (version 6.1; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer (4-15 MHz for gastrocnemii muscles or 2-10 MHz for the soleus, *biceps femoris*, and sciatic tract, Super Linear, Aix-en- Provence, France) was used in shear wave elastography mode. The region of interest (ROI) was always first identified in B-mode imaging, regardless the anatomical structure evaluated.

For the measurements performed in neural structures (sciatic nerve tract), the ultrasound transducer was positioned parallel to the nerve fibers. When used to evaluate the muscle's stiffness (*gastrocnemius medialis*, *gastrocnemius lateralis*, soleus, and *biceps femoris*), the ultrasound was positioned perpendicularly to the skin and along the muscle fascicle orientation. Specific anatomical reference points were defined to locate the target structure and then perform the elastography measurements. These points varied across studies and will be described in detail in the section corresponding to each study. Transducers were secured manually in studies 2 and 4, and secured to the thigh or the lower leg with custom-made casts (strapped to the skin) in studies 1 and 3. All the measurements were performed by me and with minimal tissue compression. The following shear wave elastography acquisition parameters were set for all the studies: penetration mode, 100% opacity, no temporal smoothing (persistence=OFF), and intermediate spatial smoothing of 5/9.

An analog trigger signal originating from the ultrasound scanner at each elastography measurement was recorded to ensure a perfect synchronization between shear wave data, ankle angle, ankle torque, and surface electromyography (EMG). In study 1, however, the trigger was detected by using a microphone coupled with the ultrasound probe (see details in the data analysis section of study 1). The maps of the shear modulus were obtained at 1 sample/s and with a spatial resolution of 1 × 1 mm.

## 5. Surface electromyography

To gather the maximal ROM, torque, and elastography measurements, myoelectrical activity was assessed using a surface EMG to assure that the lower leg muscles remained inactive during the performed ankle rotations. A pair of surface electrodes (Kendall 100 Series Foam Electrodes, Covidien, Massachusetts, USA) was placed over the *gastrocnemius medialis* (studies 1, 2, and 4),

*gastrocnemius lateralis* (studies 1, 2 and 3), soleus (studies 1, 2, 3, and 4), *biceps femoris* (study 2), and tibialis anterior (studies 1, 2, and 3) muscles at the location recommended by the Surface EMG for Non-Invasive Assessment of Muscles guidelines (Hermens et al., 2000). Some adjustments were performed on some locations of the electrodes when elastography measurements were performed on the same muscle. EMG data was acquired simultaneously with the mechanical data using the same acquisition system (see Ergometer section) at a sampling rate of 1 kHz.

## 6. Data processing

All data remained coded so that the analyses could be undertaken in a blind manner. All study data was processed using MATLAB scripts (The MathWorks Inc., Natick, USA).

### 6.1. Elastography

For all studies, both muscle and nerve stiffness estimations were based on shear wave velocity (Vs) quantification. The Vs is directly linked to the shear modulus ($\mu$) (Bercoff et al., 2004), $\mu = \rho Vs^2$, where $\rho$ is the estimated density of soft tissues (1000 kg/m$^3$). Based on this equation, results of study 1 are presented as shear modulus (kPa), whereas studies 2, 3, and 4 are presented in shear wave velocity (m/s).

Shear wave dispersion analysis has shown that shear wave propagation is guided along the Achilles tendon due to the successive reflections at the tendon boundaries. Thus, the tendon thickness is likely to influence the group shear wave velocity and, in this case, cannot be directly related to the shear modulus (Brum et al., 2014; Helfenstein-Didier et al., 2016). However, the sciatic nerve is less stiff than the Achilles tendon (i.e., measurements saturate less at low tension levels), and thus the guided shear wave propagation is likely to be less problematic (DeWall et al., 2014; Andrade et al., 2016b). Because no previous study has applied this shear wave dispersion analysis to peripheral nerves, it was decided to report the results as shear wave velocity for studies 2, 3, and 4 in which nerve stiffness was assessed (Andrade et al., 2016b).

In addition, the mean sciatic nerve thickness was calculated using the B-mode ultrasound images to ensure that pre- to post-intervention changes in shear wave velocity would not have been affected by nerve thickness adaptations to stretching (studies 3 and 4).

After data collection, the recorded videos were exported from the Aixplorer's scanner in mp4 format and then sequenced into jpeg images. The image processing converted each pixel of the color map into a shear wave velocity value based on the recorded color scale (scale = 0-18.3 m/s).

Using the MATLAB scripts, a region of interest (ROI) was firstly defined on the first map as the largest muscle or nerve area. Then, this ROI was manually tracked in the B-mode image every second during the recording performed for each ankle rotation in dorsiflexion (e.g., Figure 26 refers to elastography maps processing for the sciatic nerve). Each ROI was then inspected for artifacts (saturation areas). If artifacts were present in any of the images to be analyzed within a recording, then the ROI was reduced in size to exclude the artifact area from all images within that recording. Shear wave velocity/shear modulus data extracted from this ROI were averaged to obtain a representative value.

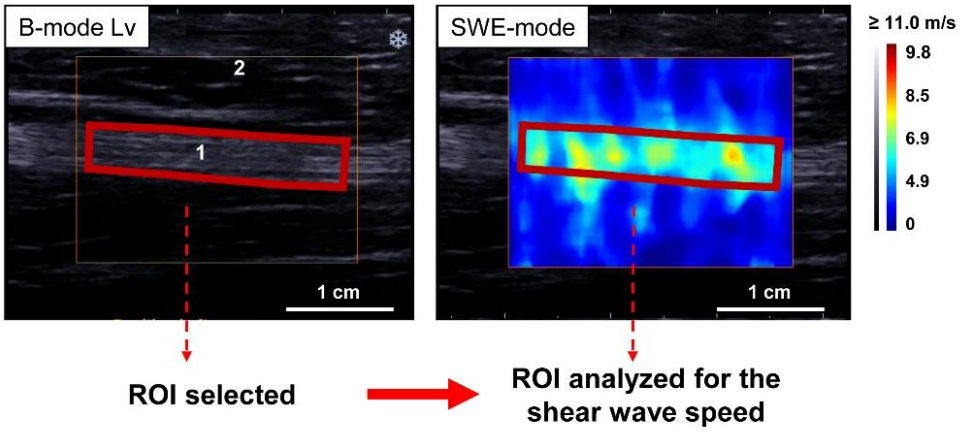


*Figure 26. (Colour outline) The ROI was determined from the B-mode image. The shear wave velocity data extracted from this ROI (SWE-mode) were averaged to obtain a representative value. Legend: 1 - Longitudinal view of sciatic nerve; 2 - Long head of biceps femoris muscle. Note: Nerve fascicles are dark (hypoechoic) and nerve superficial bounds are defined by a hyperechoic structure (epineurium).*

## 6.2. Passive joint torque-angle relationships

In all studies, angle and torque data were collected dynamically while the ankle was passively rotated from 40° in plantar flexion towards the maximal ROM in dorsiflexion. Angle and torque signals were low-pass filtered (10 Hz) with a second-order Butterworth filter. The torque was further corrected for gravity. The ankle torque was calculated every 2°, from 40° of plantar flexion to the maximal ankle ROM in dorsiflexion. Then, to standardise the ROM to a percentage of the

maximum, a linear interpolation method was used to calculate ankle torque, elastography, and EMG RMS data at every 5% of ankle ROM.

## 7. Muscular and non-muscular stretching interventions

The acute effects of non-muscular stretching (i.e., sciatic nerve stretching) were evaluated in study 3. In addition, chronic stiffness adaptations in both muscular (*triceps surae* muscles) and neural structures (sciatic nerve) were assessed in study 4, before and following either muscular or non-muscular (i.e., *triceps surae*) stretching. These stretching techniques were based on the maximal passive ROM assessments that were used to selectively load preferentially either muscular (*triceps surae*) and non-muscular structures (sciatic nerve); see sections 3.1, 3.2, and 3.3 for details.

Non-muscular stretching techniques were employed to stretch the sciatic nerve acutely (total of 6 min) and chronically (total stretch duration for the 12-week period was 7.5 h across 60 sessions) in study 3 and study 4, respectively. Lower limb posture (i.e., hip, knee and ankle angles) may significantly influence the loading of the sciatic nerve tract. Moreover, straight leg raising and slump tests are commonly used with the aim of mechanically stretching the sciatic nerve system (Breig & Braxton, 1978; Butler, 1991; Shacklock, 2005). Based on cadaver and ultrasound findings, the sciatic nerve tract was acutely and chronically stretched by manipulating the position of the hip, trunk, and cervical spine (Breig & Troup, 1979; Coppieters et al., 2006; Boyd et al., 2013; Coppieters et al., 2015). Stretching exercises were performed at the maximal tolerable hip flexion ROM and used to target the stretch of the sciatic nerve tract with minimal tension within the plantar flexors. In regard to the position of the ankle joint during testing, although previous elastography studies have demonstrated that plantar flexor muscles are minimally stretched when the ankle is in neutral position (Hirata et al., 2015; Le Sant et al., 2017), it is unlikely that such stretching magnitude is sufficient to modify the muscles' mechanical properties (Freitas et al., 2015a).

Therefore, in study 3, participants underwent a total of 6 min (2× 180 s with a 30 s rest interval) of nerve stretching. Specifically, this technique was performed by an experienced physiotherapist who passively flexed the hip from the HIP-neutral (i.e., towards a long-sitting HIP-flexed

position), followed by lumbar, thoracic, and cervical flexion to the end of ROM, as dictated by the participant (Figure 27). During this maneuver, the ankle angle was maintained in a neutral position (0°) to minimize the stretch of the plantar flexor muscles. This neural stretch technique was performed at the maximal tolerable hip flexion ROM, and was used to target the stretch of the sciatic nerve tract with minimal tension within the plantar flexors. The knee of the tested lower limb remained in full extension, while the contralateral lower limb was flexed at 90° throughout the procedure.

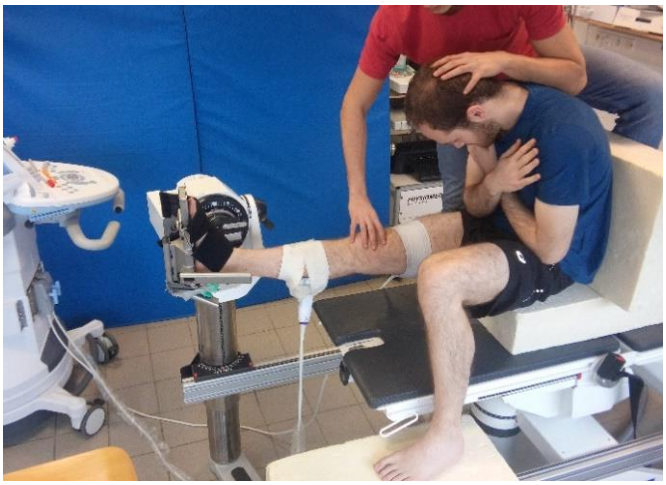

*Figure 27. Non-muscular stretching position used to preferentially stretch the sciatic nerve tract in study 3.*

Similarly to those performed in study 3, the nerve stretching exercises in study 4 were performed by experienced examiners with the aim of ensuring maximal stretch tolerance. Two sciatic stretching exercises were performed per stretching session in a randomized order: (1) from a seated position, the examiner passively flexed the hip towards a long-sitting position, followed by lumbar, thoracic, and cervical flexion to the end of ROM, as dictated by the participant. The knees remained in full extension, and the ankle angles were maintained in neutral position (0°) to minimize the stretch of the plantar flexor muscles (Figure 28-A); and (2) the participant was initially laid supine with the knees in full extension, and then participants' legs were passively raised together by an examiner (i.e., hip flexion) with the ankles in a neutral position (Figure 28-B; i.e., 0° of dorsiflexion).

In study 4, a muscular stretch targeting the plantar flexors muscles was also performed to examine the chronic effects of mechanical stimuli on tissue stiffness. Participants were instructed

to perform two self-stretching exercises in a randomized order. First, the participant was instructed to stand on a step with their heels hanging over the edge and the forefeet supporting the body weight. Then, the stretch was performed by dropping the heels slowly (both sides together) towards the ground until the maximal stretch tolerance was reached: 1) with the knees in full extension (Figure 28-C); and 2) with the knee flexed between 50° and 90° (Figure 28-D). These knee angles were chosen to specifically target both gastrocnemius (1) and soleus muscles (2), respectively (Le Sant et al., 2017). The onset of stretch pain was used as the maximal stretch tolerance for all stretches.


*Figure 28. Stretching exercises performed in study 4.* ***A.*** *and* ***B.*** *correspond to stretching exercises performed to stretch the sciatic nerve by the "sciatic STR group";* ***C.*** *and* ***D.*** are the stretching exercises performed by the "muscle STR group" to target the plantar flexors

*Chapter Four*

# EXPERIMENTAL STUDIES

# STUDY 1

## Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension

**Andrade RJ**, Lacourpaille L, Freitas SR, McNair PJ & Nordez A. (2016)

EFFECTS OF HIP AND HEAD POSITION ON ANKLE RANGE OF MOTION, ANKLE PASSIVE TORQUE, AND PASSIVE GASTROCNEMIUS TENSION

*Scandinavian journal of medicine & science in sports* 26, 41-47

# ABSTRACT

Ankle joint range of motion (ROM) is notably influenced by the position of the hip joint. However, this result remains unexplained. Thus, the aim of this study was to test if the ankle passive torque and gastrocnemius muscle tension are affected by the hip and the head positions.

The torque and the muscle shear elastic modulus (measured by elastography to estimate muscle tension) were collected in 9 participants during passive ankle dorsiflexions performed in four conditions (by combining hip flexion at 90º or 150º, and head flexed or neutral).

Ankle maximum dorsiflexion angle significantly decreased by flexing the hip from 150º to 90º ($P<0.001$; mean difference 17.7±2.5°), but no effect of the head position was observed ($P>0.05$). Maximal passive torque and shear elastic modulus were higher with the hip flexed at 90° ($P<0.001$). During submaximal ROM, no effects of the head and hip positioning ($P>0.05$) were found for both torque and shear elastic modulus at a given common ankle angle among conditions.

### *Contribution to this doctoral research:*

- Shifts in maximal dorsiflexion ROM due to hip angle position are not related neither to changes in passive ankle torque nor passive tension of the gastrocnemius.
- Further studies should be addressed to better understand the functional role of peripheral nerves and fasciae in the ankle ROM limits.
- It was decided to conduct a pilot study to explore whether ultrasound shear wave measurements could be used to assess nerve stiffness in both hip lower limb postures.

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***Keywords:*** *stretching, muscle, elastography, Supersonic shear imaging, sciatic nerve, fascia, flexibility, neurodynamics, range of motion*

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# Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension

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**Ankle joint range of motion (ROM) is notably influenced by the position of the hip joint. However, this result remains unexplained. Thus, the aim of this study was to test if the ankle passive torque and gastrocnemius muscle tension are affected by the hip and the head positions. The torque and the muscle shear elastic modulus (measured by elastography to estimate muscle tension) were collected in nine participants during passive ankle dorsiflexions performed in four conditions (by combining hip flexion at 90 or 150°, and head flexed or neutral). Ankle maximum dorsiflexion angle significantly decreased by flexing the hip from 150 to 90° ($P < 0.001$; mean difference 17.7 ± 2.5°), but no effect of the head position was observed ($P > 0.05$). Maximal passive torque and shear elastic modulus were higher with the hip flexed at 90° ($P < 0.001$). During submaximal ROM, no effects of the head and hip positioning ($P > 0.05$) were found for both torque and shear elastic modulus at a given common ankle angle among conditions. Shifts in maximal ankle angle due to hip angle manipulation are not related neither to changes in passive torque nor tension of the gastrocnemius. Further studies should be addressed to better understand the functional role of peripheral nerves and fasciae in the ankle ROM limits.**

Understanding the factors limiting range of motion (ROM) of a joint is a topic of continued interest for both researchers and clinicians. Commonly, the muscle passive tension is often estimated by using a passive torque–angle measurement (Magnusson, 1998; Gajdosik, 2001; McNair & Portero, 2005; Weppler & Magnusson, 2010). Both passive tension developed by muscle-tendon units and the sensation of this tension (i.e., discomfort) are the main limits of the maximal tolerable ROM available in a joint during a passive stretching maneuver (for review, see Weppler & Magnusson, 2010).

It is well known that the maximal dorsiflexion angle of the ankle and passive torque are affected when knee angle is changed due to a change in the gastrocnemius length (e.g., Hoang et al., 2005; Nordez et al., 2010). In respect to hip and ankle interactions, biomechanical models typically consider that these joints do not have common actuators because no muscle-tendon complex crosses both joints (Klein Horsman et al., 2007; Standring, 2008). Therefore, a change in hip angle should not affect both the passive muscle tension of ankle plantar flexors and the maximal dorsiflexion angle. "However, it has been shown that when the knee is fully extended, flexing the hip notably decreased passive maximum dorsiflexion angle of the ankle in comparison to a neutral hip position (Mitchell et al., 2008)." Two main hypotheses have been proposed to explain this finding. Firstly, a transmission of tension via lower limb fascial connections should imply an increased tension in triceps surae muscles during ankle dorsiflexion when the hip is flexed (Boyd et al., 2009). The second hypothesis is that there is a change in tension in the sciatic nerve tract. In regard to this hypothesis, there is evidence of sciatic nerve movement that is altered by trunk flexion (Ellis et al., 2012). Furthermore, Johnson and Chiarello (1997) have found a decrease in knee ROM when the head was flexed, and they proposed the tensioning of the peripheral nerves tracts as a potential mechanism. There does not appear to be similar studies that investigated the effect of cervical position on ankle ROM.

Joint torque has been extensively used to indirectly measure passive muscle mechanical properties (Magnusson, 1998; Gajdosik, 2001; McNair & Portero, 2005; Weppler & Magnusson, 2010). However, it could be argued that this parameter is more related to the resistance of the "global" musculo-articular complex to motion, and involves several anatomic structures

crossing the joint (Riemann et al., 2001). Recently, it was reported *in vitro* (Koo et al., 2013) and *in vivo* (Maisetti et al., 2012) that the measurement of the shear elastic modulus using an elastographic technique (i.e., supersonic shear imaging) is strongly related to the localized passive muscle tension during stretching. Briefly, supersonic shear imaging relies on measuring the speed of internal propagation of shear waves generated by an acoustic radiation force to estimate the shear elastic modulus of a localized area in different soft tissues (Bercoff et al., 2004). Thus, the shear elastic modulus measurement could be used to determine whether gastrocnemius tension is changed due to a change in hip angle positioning.

Therefore, the purpose of this study was to simultaneously measure the ankle torque and gastrocnemius medialis (GM) shear elastic modulus during passive ankle dorsiflexion movement through a combination of hip and head position tests. Based on previous results that show notable changes in ankle ROM of the ankle with changes in hip angle (Mitchell et al., 2008), we hypothesized that gastrocnemius elastic modulus and/or ankle joint torque could be affected by the hip positioning.

## Methods

### Participants

Nine healthy men (age: 25 ± 3 years, height: 181 ± 6 cm, weight: 73 ± 8 kg) volunteered to participate in this study and signed an informed consent form. None of the participants reported any known current or ongoing musculo-skeletal lower limb and spine injuries, neuromuscular diseases, or orthopedic-related problems. The local ethics committee approved the study, and all the procedures were undertaken in accordance with the Declaration of Helsinki.

### Equipment

#### *Ergometer*

An isokinetic dynamometer (Biodex 3 Medical, Shirley, New York, USA) was utilized to dorsiflex the ankle joint, and provide simultaneous measures of ankle angle and torque. The participants were seated in the Biodex chair with the knee fully extended (0°). The back of the chair was then adjusted to alter the hip joint position. Testing positions were determined utilizing a manual goniometer (MSD, Londerzeel, Belgium). The neutral position of the ankle was defined as 0° (i.e., foot was positioned perpendicular to the leg).

#### *Supersonic shear imaging*

An Aixplorer ultrasound scanner (version 7; 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 ware elastography mode (musculo-skeletal preset) as previously described (Bercoff et al., 2004). Assuming a linear elastic behavior, the muscle shear elastic modulus was calculated as follows (1):

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

where $\rho$ is the density of soft tissues (1000 kg/m$^3$) and $Vs$ is the shear wave speed. The region of interest for probe location was the gastrocnemius muscle belly, and this was identified by an experienced examiner. The transducer was held statically over the GM muscle belly (i.e., mid-distance between the muscle-tendon junctions) with a custom-made cast positioned perpendicularly to the skin and along the muscle fascicle orientation (Blazevich et al., 2006; Maisetti et al., 2012; Hug et al., 2013). Appropriate probe alignment was achieved when several fascicles could be traced without interruption across the image. The transducer location was not changed within the test session and therefore it is unlikely that the skin pressure induced from probe fixation could affect the trend of the muscle stiffness results during passive gastrocnemius stretching (Maisetti et al., 2012). The maps of the shear elastic modulus were obtained at 1 Hz with a spatial resolution of 1 × 1 mm.

#### *Electromyography*

To rule out any active muscle involvement, electromyography (EMG) activity was monitored during passive stretching. A pair of a conductive adhesive hydrogel surface EMG electrodes (Kendall™ 100 Series Foam Electrodes, Covidien, Massachusetts, USA) with an interelectrode distance of 20 mm (center to center) was placed over the GM, gastrocnemius lateralis, soleus, and tibialis anterior. The electrodes were located according to the recommendations of SENIAM (Surface EMG for Non-Invasive Assessment of Muscles) as described by Hermens et al. (2000). Skin was shaved and cleaned with alcohol to minimize impedance before applying the electrodes. Raw EMG signals were amplified close to the electrodes (gain 375, bandwidth 8–500 Hz) and digitized at a sampling rate of 1 kHz (ME6000, Mega Electronics Ltd., Kuopio, Finland). The EMG was monitored by an examiner during all trials.

### Protocol

The subjects were familiarized with the experimental setup. They were previously instructed not to practice vigorous exercise 48 h before the session time. The passive ankle dorsiflexion tests were performed by combinations of hip flexion at 90 or 150° with head flexion (HF) or without head flexion (HN), see Fig. 1. Thus, a total of four tests (i.e., 90 HF, 90 HN, 150 HF, 150 HN) were performed by each participant in a randomized order. A 5-min rest interval separated each test. Flexion of the head was defined as the

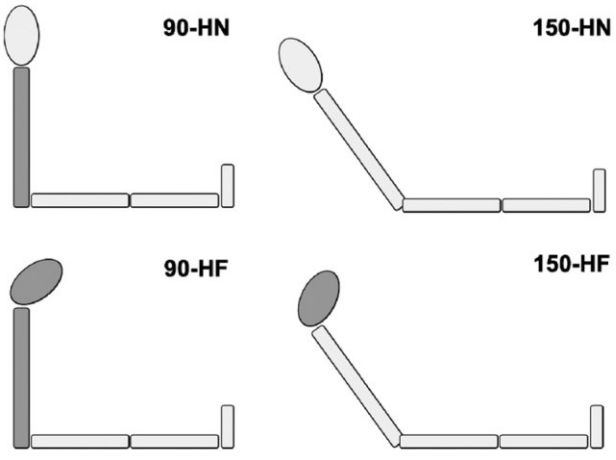


*Fig. 1.* Test conditions used in this study.
Legend: 90 HN, 90° of hip angle and head in neutral position; 90 HF, 90° of hip angle and head in flexion position; 150 HN, 150° of hip angle and head in neutral position; 150 HF, 150° of hip angle and head in flexion position.

maximum passive angle tolerated by participants and it was fixed in that position by a custom-made cast. At the beginning of each test session, five slow loading/unloading cycles (5°/s) were performed for conditioning purposes (Nordez et al., 2008). Thereafter, starting from 40° of plantar flexion, participant's plantar flexors were passively stretched (2°/s) until their maximal dorsiflexion angle was attained. The maximum perceived plantar flexor muscle stretch that participants could tolerate (i.e., onset of pain) was considered the criterion of maximal dorsiflexion ankle angle. At this point, the subjects pushed a button that immediately stopped the ankle passive motion imposed by the dynamometer. Three repetitions were done for each test condition, and the third repetition was used for all analyses. Participants were blindfolded to ensure no visual perception of stretching during testing. They were instructed to stay as relaxed as possible through each trial. Thigh and pelvis were firmly fastened with straps in all tests. EMG feedback was provided to the examiner during testing. At the end of the protocol, in order to normalize EMG-RMS (root mean square), three maximal isometric voluntary contractions were performed in plantar flexion and dorsiflexion at ankle neutral position, with 1 min of rest in-between each contraction.

### Data analysis

Data were processed using standardized Matlab (The Mathworks, Natick, Massachusetts, USA) scripts. Each focused ultrasound push beam (generated within the probe) used to produce the shear waves generates a sound signal. Thus, for each test repetition, the timing of each shear elastic modulus measurement among ankle passive motion was therefore detected using a microphone (MB Quart K800, frequency response: 40–18 000 Hz, sensitivity: 16,25 mV/Pa) coupled to the transducer, and the last timing was used to synchronize all collected data. Ankle torque was gravity corrected and calculated every second to match shear elastic modulus measurements. The RMS of electromyographic signals (RMS-EMG, averaged over 300 ms windows) was also calculated for each second. The shear elastic modulus was quantified as an average of the region of interest chosen as the biggest region without detectable artifacts defined as a localized abnormal value of the shear elastic modulus that was not present on the previous image (Bouillard et al., 2012). Maximum ankle dorsiflexion angle varied between participants. Thus, it was expressed as a percentage of the maximum ROM achieved among the four test conditions for each subject.

### Statistical analysis

The data were processed using IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, New York, USA) software. Descriptive statistics were reported as the mean and standard deviation (mean ± SD). Data were tested for normality with the Shapiro–Wilk test, and no violations were noted. Three two-way repeated measures analyses of variance (ANOVAs) (2 hip angles × 2 head angles) were used to compare absolute maximal values of ankle dorsiflexion angle, passive torque, and shear elastic modulus across test conditions. The changes in shear elastic modulus and passive torque at the largest common ankle among the four tests were compared using two two-way repeated measures ANOVAs (2 hip angles × 2 head angles). For instance, if maximum dorsiflexion angles of the 90-HF, 90-HN, 150-HF, and 150-HN tests are 20, 25, 35, and 40°, respectively, the largest common ankle angle will be 20° or 50% if it expresses as the percentage of maximum dorsiflexion ankle angle achieved among the four tests. The partial eta square ($_p\eta^2$) values were reported as measures of effect size, with moderate and large effects considered for $_p\eta^2 = 0.07$ and $_p\eta^2 \geq 0.14$, respectively (Cohen, 1988). In the present study, moderate to large effect sizes ($_p\eta^2$) were obtained across the ANOVAs performed. Because EMG-RMS did not follow a normal distribution, Friedman test was used for the mean rank comparisons between the four tested conditions for each muscle in different percentages of maximum dorsiflexion ankle angle: 20%, 40%, 60% and the highest common percentage of maximal dorsiflexion ankle angle among tests conditions (i.e., 64%). Statistical significance was set at 0.05.

## Results

A typical example of raw data (torque–angle and shear elastic modulus–angle relationships) obtained for one subject is depicted in Fig. 2.

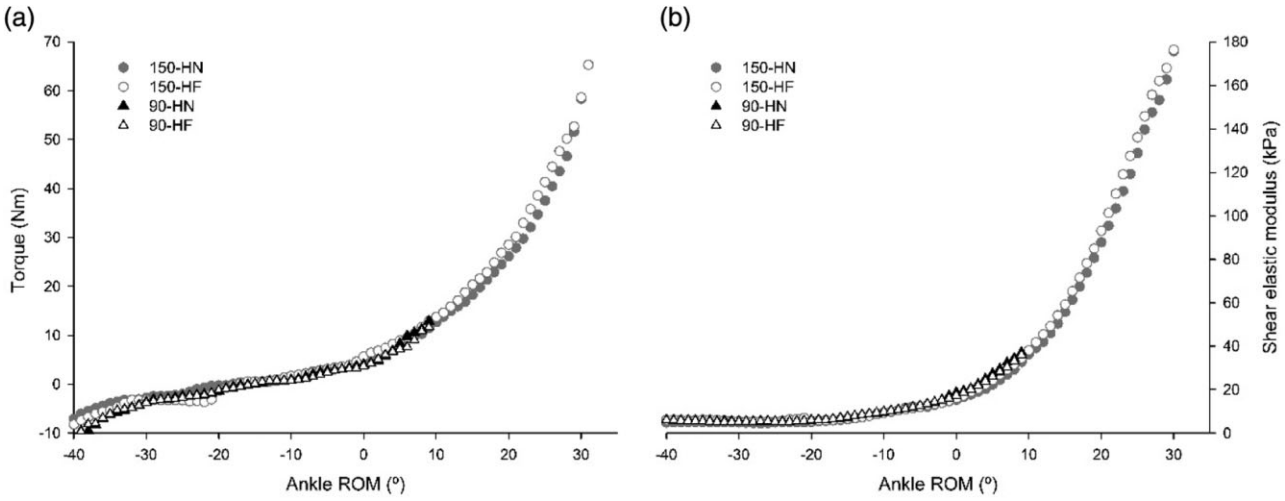


*Fig. 2.* Typical example. (a) Typical joint torque–angle curves during passive ankle dorsiflexion for each test condition. (b) Typical muscle shear elastic modulus–angle curves during passive ankle dorsiflexion for each test condition.
Legend: 90 HN, hip angle at 90° and head in neutral position; 90 HF, hip angle at 90° and head in flexion position; 150 HN, hip angle at 150° and head in neutral position; 150 HF, hip angle at 150° and head in flexion position. ROM is expressed in degrees (°).

Table 1. Average of absolute values of torque and shear elastic modulus at endpoint of maximum stretching and maximum ROM accepted by participants in each test condition

| | Test condition | | | |
|---|---|---|---|---|
| | 90 HN | 90 HF | 150 HN | 150 HF |
| Maximum dorsiflexion angle of the ankle (°) | 19.11 ± 7.20 | 18.89 ± 6.92 | 36.62 ± 6.45 | 36.71 ± 6.31 |
| Peak torque (Nm) | 30.31 ± 13.42 | 30.10 ± 18.39 | 69.53 ± 14.02 | 67.37 ± 12.46 |
| Shear elastic modulus (kPa) | 71.46 ± 24.50 | 70.36 ± 31.54 | 140.41 ± 38.97 | 143.26 ± 41.85 |

Values are presented as mean ± SD. Legend: 90 HN, hip angle at 90° and head in neutral position; 90 HF, hip angle at 90° and head in flexion position; 150 HN, hip angle at 150° and head in neutral position; 150 HF, hip angle at 150° and head in flexion position. ROM, range of motion; 0° = neutral position of the ankle joint (foot positioned perpendicular to the leg).

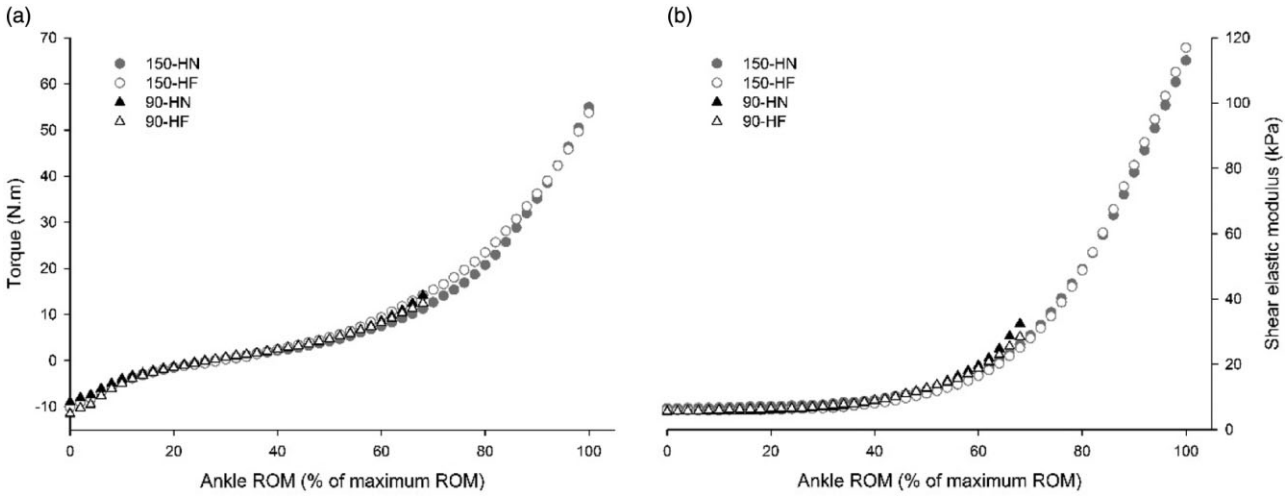


*Fig. 3.* Average results. (a) Average joint torque–angle curves during passive ankle dorsiflexion for each test condition. (b) Average muscle shear elastic modulus–angle curves during passive ankle dorsiflexion for each test condition.
Legend: 90 HN, hip angle at 90° and head in neutral position; 90 HF, hip angle at 90° and head in flexion position; 150 HN, hip angle at 150° and head in neutral position; 150 HF, hip angle at 150° and head in flexion position. ROM is expressed as a percentage of the maximum ROM achieved in the overall four test conditions by each subject. *No significant differences ($P < 0.05$) were observed for every common ROM between conditions.

### Absolute maximum ankle angle, shear elastic modulus, torque, and EMG

A significant main effect of hip angle was observed for the following dependent variables: maximal ankle angle (mean difference = 17.67 ± 2.48, $P < 0.001$; ${}_p\eta^2 = 0.89$), peak torque (mean difference = 38.25 ± 4.65 Nm, $P < 0.001$; ${}_p\eta^2 = 0.95$), and peak shear elastic modulus (+69.00 ± 14.29 kPa, $P = 0.001$; ${}_p\eta^2 = 0.90$) (Table 1). No main effects or interactions associated with head position were observed for all dependent variables ($P > 0.05$; ${}_p\eta^2 > 0.14$). No main effects or interactions were found for EMG-RMS of each muscle (mean %MVC EMG signals across muscles were less than 2% and did not vary across the entire ROM).

### Passive torque–angle relationship

No hip and head position effects or interactions were observed throughout comparable ankle ROM (Fig. 3(a); $P > 0.05$; $p\eta^2 > 0.07$ for all ankle angles until 64% of maximal dorsiflexion).

### Muscle shear elastic modulus–angle relationship

Averaged curves of the shear elastic modulus–angle relationships across subjects are shown in Fig. 3. No hip and head effects were observed among comparable ankle angles until the highest common ankle angle among performed tests ($P > 0.05$; ${}_p\eta^2 = 0.08$ for head effects and ${}_p\eta^2 > 0.14$ for hip effects and interactions).

## Discussion

This study describes the effect of the hip and the head positions on the ankle joint dorsiflexion ROM limit, the ankle torque, and the GM passive tension estimated using the shear elastic modulus measurement. No previous study has simultaneously investigated these parameters. The main finding was that maximum dorsiflexion angle of the ankle was strongly affected by the hip angle position, while ankle torque and passive tension of the GM were unchanged for an equivalent ankle angle. In addition, the head position did not affect the ankle dorsiflexion ROM.

A dramatic decrease in maximum dorsiflexion angle of the ankle (> 50%) was found when hip angle was changed from 150 to 90°. This result is in accordance with the study of Mitchell et al. (2008). They have demonstrated that the hip flexion with the knee fully extended produced significant deficits in ankle joint ROM (ΔROM 22.3°, 95% CI 18.0–26.7). Other studies also support these results. For instance, Gajdosik et al. (1985) reported a decrease ($10.1 \pm 5.1°$) in hip maximum ROM when performing a passive straight leg raise (SLR) with the ankle in a dorsiflexed position compared with an SLR performed with the ankle in plantar flexion. Boyd et al. (2009) have observed quite similar results (decrease in $10.1 \pm 9.7°$) for the same tests. However, none of these previous studies have investigated other important variables (e.g., passive torque).

Our results clearly demonstrate that both the ankle passive torque and the GM shear elastic modulus obtained for a given common ankle angle are unaffected by the hip and the head positions (Fig. 3). While several structures contribute to the passive torque, elastographic measurements performed in the present study were focused on one targeted muscle and provide an indirect estimation of its passive muscle tension (Maisetti et al., 2012). Thus, our results suggest that ROM changes were not caused by alterations in GM muscle tension. However, the specific influence of other minor plantar flexor muscles should be examined in the future. In addition, the peak torque and the maximal shear elastic modulus obtained at the maximal ROM were significantly higher when the hip was positioned at 150° (Table 1). Thus, the participants were able to achieve much more tension in the plantar flexor muscles with the hip extended compared with the hip flexed because in the first condition (hip extended) they tolerated higher dorsiflexion ROM.

Our results also suggest that there is no force transmission between hip-related structures and the gastrocnemius muscle. Therefore, other anatomical structures that cross both the hip and the ankle joints that do not influence ankle torque should explain the decrease in maximal dorsiflexion angle and stretch tolerance when the hip is flexed.

Fascia and peripheral nerves are passive and continuous anatomical structures that cross both hip and ankle joints, and their contributions to the limitations of the maximal dorsiflexion ankle angle when the hip is flexed have not been investigated. The architecture of the superficial and deep fascia system of the lower limb, between ankle and hip joints, has been documented (Gerlach & Lierse, 1990). However, the role of fascial tissue on force transmission between non-mechanically-related joints and muscle-tendon complexes during passive stretching remains unknown. Carvalhais et al. (2013) noted that passive torque is increased when fascial tissue is indirectly tensioned by moving a non-related joint. This suggests that the tension of the fascial tissue may affect the passive torque and muscle tension. In the current study, the lack of changes in torque and shear elastic modulus for a given ankle angle suggests that fasciae were not involved notably in the changes in dorsiflexion ROM across test conditions.

In regard to the effect of peripheral nerves, it seems improbable that a change in nerve tension may induce significant changes in the passive torque, but it may affect stretch tolerance. The biomechanical behavior of peripheral nerves has been studied (Topp & Boyd, 2006; Silva et al., 2014). Some studies have observed that both hip flexion (Coppieters et al., 2006; Ridehalgh et al., 2014) and ankle dorsiflexion (Coppieters et al., 2006; Alshami et al., 2008) substantially increased nerve deformation. In addition, Boyd et al. (2012) highlighted this rationale by showing a mean threefold reduction in the tibial nerve distal movement during ankle dorsiflexion when the hip was flexed. Furthermore, in a study involving cadavers, Borrelli et al. (2000) showed that intraneural pressure increased significantly when the hip was flexed from 0 to 45° of flexion and again when the hip was brought from 45 to 90° of flexion. The intraneural tissue fluid pressures measured within a localized section of the sciatic nerve appeared to exceed published critical thresholds for alterations of blood flow and neural function only when the hip was flexed to 90° and the knee was fully extended. Thus, the earlier stretching endpoint observed in our study when the hip was flexed with the knee fully extended could be explained by the increased mechanosensitivity of peripheral nerves. This is thought to be a normal protective response to the stresses applied to nerves during limb movement (Boyd et al., 2009). While this is a plausible possibility, there could also be other neural mechanisms that result in increased perception of tension or end range sensation. For instance, increased tension in a muscle group (e.g., muscles crossing hip and knee) might cause inhibition-excitation on sensory pathways through various interneurons.

In respect to the EMG results, these suggest that the changes in ROM observed were unlikely to have been influenced by local muscle activity. The low values of EMG activity in our study were in accordance with previous work (McNair et al., 2001, 2002; Nordez et al., 2010). More importantly, no hip and head effects were observed across ROM. In addition, the stretching repetitions performed in the present study should influence the passive torque and shear elastic modulus. It is classically considered that 5 min of rest is sufficient to counteract the effects of five cyclic stretching repetitions at slow velocity (Nordez et al., 2008, 2009). Moreover, the order of testing was randomized. Therefore, while the possibility of stretching effects can not be excluded, we think that it did not influence our results.

The present study also showed that the maximal ankle dorsiflexion angle, torque, and muscle tension of GM were unaffected by the head position, thus providing

some evidence that the structure that limited ankle ROM was not strongly linked to the cervical spine. Similarly, Ellis et al. (2012) showed that minimal sciatic nerve excursion was evident during isolated cervical flexion.

In conclusion, our results suggest that less typically reported structures that cross both the hip and the ankle joint contribute to the ROM at the latter joint. Although our findings provide a better understanding of inter-joint biomechanical behavior, further investigations are required to explore the mechanisms behind this finding. In particular, the use of elastographic methods could provide interesting information about the role of peripheral nerve mechanics on joint motion.

## Perspectives

Different hip angle positions notably affect the maximal ankle dorsiflexion angle, while the ankle torque and passive tension of the GM are unchanged for a given common ankle angle. The angle of the head did not cause any further change in ankle dorsiflexion ROM. This knowledge regarding the varied amount of ankle angle observed without any further changes in muscle tension and ankle torque should be important to aid in the design of lower limb manual therapy exercises, such as neurodynamic tests, and diagnosis maneuvers that simultaneously use ankle dorsiflexion and different hip angles (e.g., SLR test). Furthermore, the findings of this study suggest that both peripheral nerves and fasciae should be studied in the future to better understand their role in the ROM limits of the ankle joint. This study was limited to participants with no history of muscle-tendon, articular, or nerve pathologies. The same kind of experiment could be valuable to analyze the factors that affect the ROM limits in individuals with neuromuscular disorders.

**Key words:** Stretching, muscle, elastography, supersonic shear imaging, sciatic nerve, fascia, flexibility, neurodynamics, range of motion.

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## References

Alshami AM, Babri AS, Souvlis T, Coppieters MW. Strain in the tibial and plantar nerves with foot and ankle movements and the influence of adjacent joint positions. J Appl Biomech 2008: 24: 368–376.

Bercoff J, Tanter M, Fink M. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. IEEE Trans Ultrason Ferroelectr Freq Control 2004: 51: 396–409.

Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. J Anat 2006: 209: 289–310.

Borrelli J Jr, Kantor J, Ungacta F, Ricci W. Intraneural sciatic nerve pressures relative to the position of the hip and knee: a human cadaveric study. J Orthop Trauma 2000: 14: 255–258.

Bouillard K, Hug F, Guevel A, Nordez A. Shear elastic modulus can be used to estimate an index of individual muscle force during a submaximal isometric fatiguing contraction. J Appl Physiol 2012: 113: 1353–1361.

Boyd BS, Gray AT, Dilley A, Wanek L, Topp KS. The pattern of tibial nerve excursion with active ankle dorsiflexion is different in older people with diabetes mellitus. Clin Biomech (Bristol, Avon) 2012: 27: 967–971.

Boyd BS, Wanek L, Gray AT, Topp KS. Mechanosensitivity of the lower extremity nervous system during straight-leg raise neurodynamic testing in healthy individuals. J Orthop Sports Phys Ther 2009: 39: 780–790.

Carvalhais VO, Ocarino Jde M, Araujo VL, Souza TR, Silva PL, Fonseca ST. Myofascial force transmission between the latissimus dorsi and gluteus maximus muscles: an in vivo experiment. J Biomech 2013: 46: 1003–1007.

Cohen J. Statistical power analysis for the behavioural sciences. New York: Academic Press, 1988: 273–406.

Coppieters MW, Alshami AM, Babri AS, Souvlis T, Kippers V, Hodges PW. Strain and excursion of the sciatic, tibial, and plantar nerves during a modified straight leg raising test. J Orthop Res 2006: 24: 1883–1889.

Ellis RF, Hing WA, McNair PJ. Comparison of longitudinal sciatic nerve movement with different mobilization exercises: an in vivo study utilizing ultrasound imaging. J Orthop Sports Phys Ther 2012: 42: 667–675.

Gajdosik RL. Passive extensibility of skeletal muscle: review of the literature with clinical implications. Clin Biomech (Bristol, Avon) 2001: 16: 87–101.

Gajdosik RL, LeVeau BF, Bohannon RW. Effects of ankle dorsiflexion on active and passive unilateral straight leg raising. Phys Ther 1985: 65: 1478–1482.

Gerlach UJ, Lierse W. Functional construction of the superficial and deep fascia system of the lower limb in man. Acta Anat 1990: 139: 11–25.

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 2000: 10: 361–374.

Hoang PD, Gorman RB, Todd G, Gandevia SC, Herbert RD. A new method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. J Biomech 2005: 38: 1333–1341.

Hug F, Lacourpaille L, Maisetti O, Nordez A. Slack length of gastrocnemius medialis and Achilles tendon occurs at different ankle angles. J Biomech 2013: 46 (14): 2534–2538.

Johnson EK, Chiarello CM. The slump test: the effects of head and lower extremity position on knee extension. J Orthop Sports Phys Ther 1997: 26: 310–317.

Klein Horsman MD, Koopman HF, van der Helm FC, Prose LP, Veeger HE. Morphological muscle and joint parameters for musculoskeletal modelling of the lower extremity. Clin Biomech (Bristol, Avon) 2007: 22: 239–247.

Koo TK, Guo JY, Cohen JH, Parker KJ. Relationship between shear elastic modulus and passive muscle force: an ex-vivo study. J Biomech 2013: 46: 2053–2059.

Magnusson SP. Passive properties of human skeletal muscle during stretch maneuvers. A review. Scand J Med Sci Sports 1998: 8: 65–77.

Maisetti O, Hug F, Bouillard K. Nordez A. Characterization of passive elastic properties of the human medial gastrocnemius muscle belly using supersonic shear imaging. J Biomech 2012: 45: 978–984.

McNair PJ, Dombroski EW, Hewson DJ, Stanley SN. Stretching at the ankle joint: viscoelastic responses to holds and continuous passive motion. Med Sci Sports Exerc 2001: 33: 354–358.

McNair PJ, Hewson DJ, Dombroski E, Stanley SN. Stiffness and passive peak force changes at the ankle joint: the effect of different joint angular velocities. Clin Biomech (Bristol, Avon) 2002: 17: 536–540.

McNair PJ, Portero P. Using isokinetic dynamometers for measurements associated with tissue extensibility. Isokinet Exerc Sci 2005: 13: 53–56.

Mitchell B, Bressel E, McNair PJ, Bressel ME. Effect of pelvic, hip, and knee position on ankle joint range of motion. Phys Ther Sport 2008: 9: 202–208.

Nordez A, Foure A, Dombroski EW, Mariot JP, Cornu C, McNair PJ. Improvements to Hoang et al.'s method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. J Biomech 2010: 43: 379–382.

Nordez A, McNair P, Casari P, Cornu C. Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. Int J Sports Med 2008: 29: 414–418.

Nordez A, McNair PJ, Casari P, Cornu C. The effect of anular velocity and cycle on the dissipative properties of the knee during passive cyclic stretching: a matter of viscosity or solid friction. Clin Biomech 2009: 24: 77–81.

Ridehalgh C, Moore A, Hough A. Normative sciatic nerve excursion during a modified straight leg raise test. Man Ther 2014: 19: 59–64.

Riemann BL, DeMont RG, Ryu K, Lephart SM. The effects of sex, joint angle, and the gastrocnemius muscle on passive ankle joint complex stiffness. J Athl Train 2001: 36: 369–375.

Silva A, Manso A, Andrade R, Domingues V, Brandao MP, Silva AG. Quantitative in vivo longitudinal nerve excursion and strain in response to joint movement: a systematic literature review. Clin Biomech (Bristol, Avon) 2014: 29 (8): 839–847. doi: 10.1016/j.clinbiomech.2014.07.006

Standring S. Gray's anatomy: the anatomical basis of clinical practice. Philadelphia, PA: Churchill Livingstone/Elsevier, 2008: 468–604.

Topp KS, Boyd BS. Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. Phys Ther 2006: 86: 92–109.

Weppler CH, Magnusson SP. Increasing muscle extensibility: a matter of increasing length or modifying sensation? Phys Ther 2010: 90: 438–449.

# PILOT STUDY

Effects of hip position on sciatic nerve stiffness

Following the study 1, a pilot study was conducted to explore whether ultrasound shear wave elastography could be used to measure dynamically the nerve stiffness and, thus, to address the hypothesis raised about the mechanical role of peripheral nerves in the limitation of maximal joint ROM. Specifically, local stiffness measurements could provide valuable and direct information about the individual mechanical role of sciatic nerve tract in the limitation of maximal dorsiflexion ROM when flexing the hip joint from a neutral (HIP-neutral) to a more flexed position (HIP-flexed).

The stiffness of sciatic nerve (at mid-thigh level) and its tibial branch (at lower leg level) and *gastrocnemius medialis* was measured in two lower limb postures: HIP-neutral (Figure 1-A) and HIP-flexed (Figure 1-B).

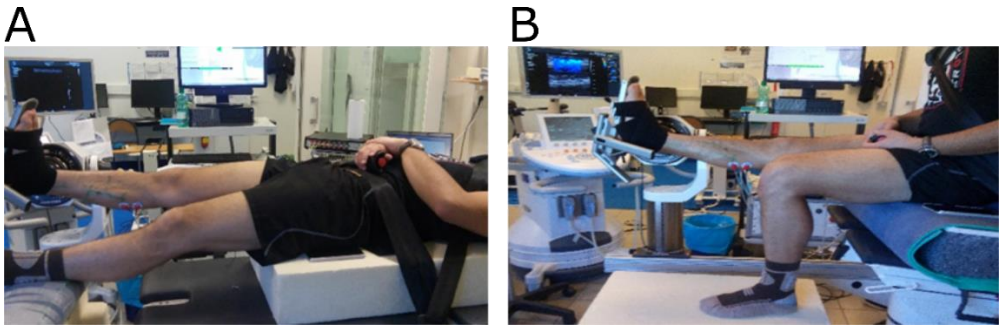


*Figure 1. Postures used to assess the stiffness of the sciatic nerve tract (at mid-thigh level, and its tibial branch at lower leg) during passive maximal dorsiflexion range of motion assessments.* ***A.*** *Hip is positioned in neutral position (i.e. 180°) while the knee is fully extended.* ***B.*** *Hip is flexed at 90° while the knee is remained in a full extension position.*

Our hypothesis were defined in accordance with the findings of the first study of this doctoral research, which suggested that the stiffness of sciatic nerve tract: 1) would increase considerably in the nerve structure without affecting the passive tension of *gastrocnemius medialis*; and 2) the addition of progressive ankle dorsiflexion will increase both muscles and nerves stiffness. For that, fourteen healthy volunteers participated in this pilot study (8 males and 4 females; age: 22.6 ± 1.6 years, height: 171.4 ± 3.7 cm, weight: 63.6 ± 5.2 kg).

Briefly, the results of this pilot study allowed to conclude that nerve stiffness (both sciatic and its tibial branch) increase significantly with the flexion of the hip (Figure 2). This finding strongly suggests that HIP-flexed posture stretches the sciatic nerve tract in the absence of changes in *gastrocnemius medialis*.

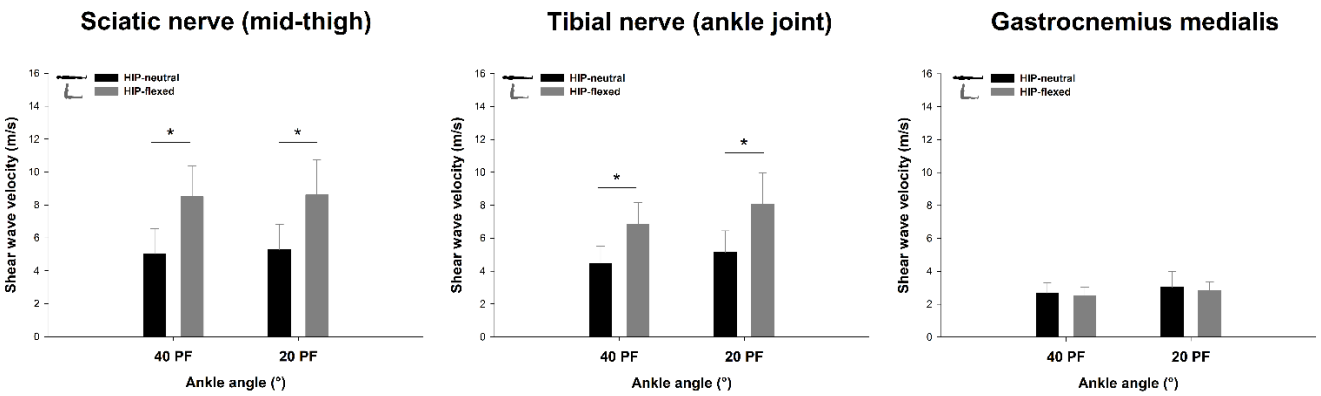


*Figure 2. Effects of hip position on shear wave velocity of sciatic nerve and its tibial branch (tibial nerve) and gastrocnemius medialis muscle. Measurements were performed at 40° and 20° of plantar flexion (PF). * P<0.05; analysis of variance showed that sciatic nerve shear wave velocity increase significantly when flexing the hip.*

However, high levels of saturation (e.g. Figure 3) were detected within the nerve boundaries at relatively low ankle angles (i.e. 20/10° in plantar flexion) for the HIP-flexed position (Figure 1-B). The saturation further increased as the ankle was passively dorsiflexed towards maximal dorsiflexion ROM. Thus, when tested in HIP-flexed the saturation limit of the elastographic scanner (i.e. 18.3 m/s) is reached in plantar flexion.

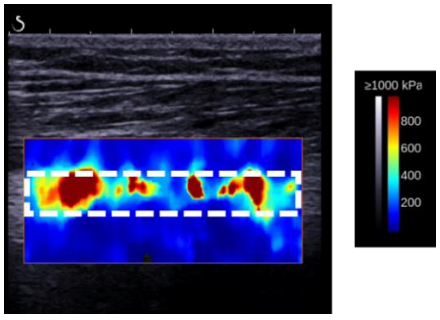


*Figure 3. Example of a saturated measurement in sciatic nerve (mid-thigh level). The measurement refers to a HIP-flexed position with the ankle positioned at 20° of plantar flexion. Saturated region corresponds to red regions*

In contrast, the nerve stiffness measurements performed in HIP-neutral did not saturate during the entire ankle ROM assessments (i.e. from 40° in plantar flexion to maximal dorsiflexion ROM). Two main conclusions emerged. Firstly, the mechanical behaviour of the sciatic nerve to stretch cannot be assessed in HIP-flexed by using ultrasound shear wave elastography. Secondly, the sciatic nerve may load during passive dorsiflexion performed in HIP-neutral and, overall, we obtained good maps of shear wave measurements.

# STUDY 2

## Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography

**Andrade RJ**, Nordez A, Hug F, Ates F, Coppieters MW, Pezarat-Correia P & Freitas SR. (2016)

NON-INVASIVE ASSESSMENT OF SCIATIC NERVE STIFFNESS DURING HUMAN ANKLE MOTION USING ULTRASOUND SHEAR WAVE ELASTOGRAPHY

*Journal of biomechanics 49, 326-33*

# ABSTRACT

Peripheral nerves are exposed to mechanical stress during movement. However the in vivo mechanical properties of nerves remain largely unexplored. The primary aim of this study was to characterize the effect of passive dorsiflexion on sciatic nerve shear wave velocity (an index of stiffness) when the knee was in 90° flexion (knee 90º) or extended (knee 180º). The secondary aim was to determine the effect of five repeated dorsiflexions on the nerve shear wave velocity.

Nine healthy participants were tested. The repeatability of sciatic nerve shear wave velocity was good for both knee 90° and knee 180° (ICCs $\geq$ 0.92, CVs $\leq$ 8.1%). The shear wave velocity of the sciatic nerve significantly increased ($p < 0.0001$) during dorsiflexion when the knee was extended (knee 180°), but no changes were observed when the knee was flexed (90º). The shear wave velocity-angle relationship displayed a hysteresis for knee 180º. Although there was a tendency for the nerve shear wave velocity to decrease throughout the repetition of the five ankle dorsiflexions, the level of significance was not reached ($p = 0.055$).

### *Contribution to this doctoral research:*

- The sciatic nerve stiffness can be non-invasively assessed during passive movements;
- These results highlight the importance of considering both the knee and the ankle position for clinical and biomechanical assessment of the sciatic nerve.

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***Keywords:*** *Supersonic shear imaging; Shear wave velocity; Peripheral nerves; Sciatic nerve; Noninvasive mechanics*

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# Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography

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ABSTRACT

Peripheral nerves are exposed to mechanical stress during movement. However the *in vivo* mechanical properties of nerves remain largely unexplored. The primary aim of this study was to characterize the effect of passive dorsiflexion on sciatic nerve shear wave velocity (an index of stiffness) when the knee was in 90° flexion (knee 90°) or extended (knee 180°). The secondary aim was to determine the effect of five repeated dorsiflexions on the nerve shear wave velocity. Nine healthy participants were tested. The repeatability of sciatic nerve shear wave velocity was good for both knee 90° and knee 180° (ICCs $\geq$ 0.92, CVs $\leq$ 8.1%). The shear wave velocity of the sciatic nerve significantly increased ($p < 0.0001$) during dorsiflexion when the knee was extended (knee 180°), but no changes were observed when the knee was flexed (90°). The shear wave velocity–angle relationship displayed a hysteresis for knee 180°. Although there was a tendency for the nerve shear wave velocity to decrease throughout the repetition of the five ankle dorsiflexions, the level of significance was not reached ($p = 0.055$). These results demonstrate that the sciatic nerve stiffness can be non-invasively assessed during passive movements. In addition, the results highlight the importance of considering both the knee and the ankle position for clinical and biomechanical assessment of the sciatic nerve. This non-invasive technique offers new perspectives to provide new insights into nerve mechanics in both healthy and clinical populations (e.g., specific peripheral neuropathies).



## 1. Introduction

Peripheral nerves are exposed to significant mechanical stress during movement as they elongate and slide relative to adjacent tissues (Silva et al., 2014; Topp and Boyd, 2006). Furthermore, nerve excursion and strain are altered in people with peripheral neuropathies (Boyd and Dilley, 2014; Hough et al., 2007). As impaired nerve biomechanics is associated with compromised nerve function (Jou et al., 2000; Li and Shi, 2007; Rickett et al., 2011), a non-invasive quantification of the mechanical properties of peripheral nerves during movement may provide valuable information for the diagnosis and management of people with peripheral neuropathies.

The mechanical properties of peripheral nerves have been extensively examined in animal models (Kwan et al., 1992; Millesi et al., 1995), human cadaver studies (Boyd et al., 2013; Coppieters et al., 2006) and human *in vivo* studies (Boyd et al., 2012; Ellis et al., 2012). The majority of *in vivo* studies used ultrasound imaging to quantify nerve excursion and morphology (e.g., cross sectional area) (Beekman and Visser, 2004; Dilley et al., 2001; Silva et al., 2014). However, nerve excursion cannot be used to infer changes in stiffness or force. This is because the nerves may experience changes in their length within a slack length range (i.e., toe region of the load–elongation curve) while their stiffness or tension does not vary (Bueno and Shah, 2008; Coppieters and Butler, 2008; Topp and Boyd, 2006). Because of this limitation, the effects of joint motion on nerve stiffness and on tensile loads are largely unknown in *in vivo* situations. The influence of positions of neighbouring joints on nerve stiffness and tensile load

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also remains largely unexamined. Finally, it is unclear whether repetitive loading or conditioning of the nerve affects its mechanical properties as is classically observed for other soft tissues such as skeletal muscles (McNair et al., 2002) and tendons (Maganaris, 2003).

Ultrasound shear wave elastography techniques, such as Supersonic Shear Imaging (SSI), measure the shear wave velocity within soft tissues (Bercoff et al., 2004). The shear wave velocity is directly linked to the shear modulus (Bercoff et al., 2004) and, thereby, can be used as a non-invasive metric of tissue stiffness (DeWall et al., 2014) and passive tension (Eby et al., 2013; Koo et al., 2013; Maisetti et al., 2012).

The primary aim of the present study was to characterize the effect of passive dorsiflexion on sciatic nerve shear wave velocity (an index of stiffness) when the knee was in 90° flexion (knee 90°) or extended (knee 180°). The secondary aim was to determine the effect of five repeated dorsiflexions on the nerve stiffness. To address these aims, we first needed to assess the reliability of shear wave velocity measurements for the sciatic nerve. Due to its particular mechanical relationship with knee and ankle joints, we hypothesized that the sciatic nerve stiffness would increase during both ankle dorsiflexion and knee extension.

## 2. Methods

### 2.1. Participants

Ten healthy males without low back or leg pain (mean (SD); age: 25.3 (2.5) years, height: 181.8 (6.9) cm, weight: 75.6 (7.5) kg) volunteered to participate in this study. Healthy individuals were selected as our intention was to reveal normal nerve biomechanics and to eliminate potentially confounding variables associated with dysfunction. Participants had no history of significant trauma or surgery to the spine, hip or hamstring region, or symptoms consistent with sciatic or tibial nerve pathology (e.g., pain, paraesthesias or weakness). The slump test [a test to assess the mechanosensitivity of the nervous system (Butler, 2000)] was negative in all participants. Participants had no known systemic disorders (e.g., diabetes) or signs of neurological conditions that might alter the function or morphology of the peripheral nervous system. The local ethics committee approved the study, and all the procedures were in agreement with the Declaration of Helsinki. Informed consent was obtained from all participants.

### 2.2. Experimental conditions

Shear wave velocity was measured within the sciatic nerve during passive ankle dorsiflexion with: *i*) knee in full extension (i.e., knee 180°); and *ii*) with the knee in 90° flexion (i.e., knee 90°). The ankle was passively moved from 40° of plantarflexion to 80% of the maximal range of dorsiflexion. The maximal range of dorsiflexion was defined as the angle corresponding to the onset of pain induced by the stretch of the plantar flexors.

An isokinetic dynamometer (Biodex 3 Medical, Shirley, NY, USA) was used to impose the ankle movements and determine the maximal range of motion (ROM). The neutral position (i.e., 0° of the ankle) was defined as an angle of 90° between the footplate of the dynamometer and the shank (i.e., foot perpendicular to the leg). This position was determined using an inclinometer. The lateral malleolus was aligned with the axis of the dynamometer and was considered as an estimate of the axis of rotation. Ankle angle data were collected at 1 kHz with an analogue/digital converter (ADInstruments, Powerlab 16/35, New Zealand). Positions of the hip (i.e., neutral) and knee were verified using a standard goniometer (MSD, Londerzeel, Belgium).

### 2.3. Elastography measurements

Shear wave velocity maps of the sciatic nerve were measured with an Aixplorer ultrasound scanner (Version 6.1; Supersonic Imagine, Aix-en-Provence, France) in shear wave elastography (SWE) mode (Bercoff et al., 2004) using the musculoskeletal (MSK) pre-set. A linear transducer (SL 10–2, Supersonic Imagine, Aix-en-Provence, France) was used. There was no temporal smoothing (persistence = OFF) and an intermediate spatial smoothing (5/9) was used. The maps of the shear wave velocity (Fig. 1, SWE-mode) were obtained at 1 sample/s with a spatial resolution of 1 × 1 mm.

First, the sciatic nerve was identified in the right posterior thigh ~7–10 cm distal to the gluteal fold using B-mode images (Bruhn et al., 2009, 2008; Karmakar et al., 2007). The probe was positioned perpendicular to the course of the sciatic nerve to obtain a cross sectional image (Fig. 1; B-mode Tv). At this level, the sciatic nerve is commonly an asymmetric structure. In order to obtain a representative value, the probe was centred over the thickest part of the nerve. The transducer was then rotated (90°) to acquire a longitudinal view of the sciatic nerve, and the SWE-mode was set. All ultrasound measurements were performed by an experienced examiner.

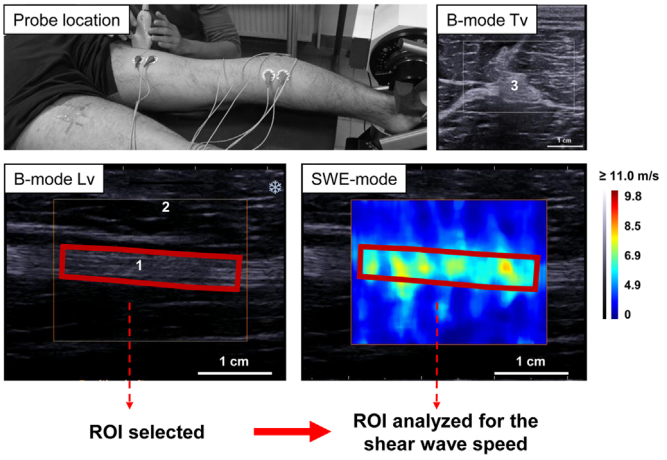


**Fig. 1.** Acquisition and processing of the maps of shear wave speed, First, the sciatic nerve was identified in the right posterior thigh ~7–10 cm distal to the gluteal fold using B-mode images. The probe was positioned perpendicular to the course of the sciatic nerve to obtain a cross sectional image (B-mode Tv). The transducer was then rotated (90°) to acquire a longitudinal view of the sciatic nerve (B-mode Lv). The region of interest (ROI) was determined from the B-mode image. The shear wave velocity data extracted from this ROI (SWE-mode) were averaged to obtain a representative value, legend: 1-Longitudinal view of sciatic nerve; 2-Long head of biceps femoris muscle. 3-Transverse view of sciatic nerve. Note: nerve fascicles are dark (hypoechoic) and nerve superficial bounds are defined by a hyperechoic structure (epineurium).

The location of the transducer was marked on the skin using a waterproof marker so that the transducer location remained constant across the tasks. A semi-rigid cable supported the manually held ultrasound transducer to reduce a possible motion of the transducer during the measurements.

Note that the shear wave velocity is reported in the present study, rather than the shear modulus as in previous muscle studies (Eby et al., 2013; Koo et al., 2013; Maisetti et al., 2012). In an infinite medium, the shear modulus ($\mu$) can be directly calculated from the shear wave speed ($Vs$) using the following equation (Bercoff et al., 2004):

$$\mu = \rho V_s^2 \quad (1)$$

where $\rho$ is the density of soft tissues (1000 k/gm$^3$). Using a shear wave dispersion analysis applied to Achilles tendon, Brum et al. (2014) showed that the shear wavelength (directly linked to the shear wave velocity) is longer than the tendon thickness. Therefore the wave propagation is guided because the medium cannot be considered as infinite. For this reason, recent tendon studies using SSI have reported shear wave velocity (DeWall et al., 2014; Slane et al., 2015). As shown in the present study, nerves are much less stiff than tendons and thus this issue is likely to be less problematic. However, as no study has applied the shear wave dispersion analysis to nerves, we chose to present our results as shear wave velocity.

### 2.4. Electromyography

Surface electromyography (EMG) was recorded to ensure that muscles were inactive during the elastography measurements. Surface electrodes (Kendall™ 100 Series Foam Electrodes, Covidien, Massachusetts, USA) were placed over the medial and lateral part of the gastrocnemius (MG and LG, respectively), tibialis anterior (TA), soleus (SOL) and semitendinosus (ST) muscles. Electrodes were placed according to the SENIAM recommendations [Surface EMG for Non-Invasive Assessment of Muscles (Hermens et al., 2000)] with an inter-electrode distance of 20 mm. Signals were amplified ( × 1000) and digitized (8–500 Hz bandwidth) at a sampling rate of 1 kHz (ADInstruments, Powerlab 16/35, New Zealand). The root mean square of EMG signals (RMS-EMG, averaged over a time window of 300 ms) was calculated every seconds. Then, the RMS-EMG values were normalized to that recorded during maximal isometric voluntary contraction (MVIC).

### 2.5. Data collection and analysis

Five conditioning cycles of passive ankle dorsiflexion and plantarflexion were first performed. These trials were conducted to evaluate a possible conditioning effect of the ankle movements on sciatic nerve mechanical properties during the initial loading–unloading cycles. The conditioning cycles were performed at 5°/s. Three post-conditioning loading and unloading motions were performed at 2°/s to measure the effect of ankle position on sciatic nerve stiffness and to calculate the reliability of the nerve shear wave speed measurement. The measurements were

randomly performed with the knee in flexion (knee 90°) and extension (knee 180°), and there was a 5-min rest period between experimental conditions.

Maps of the shear wave velocity were processed using a custom Matlab script (The Mathworks, Natick, USA). The region-of-interest (ROI) was first determined in the B-mode image by selecting the largest area in accordance with nerve boundaries (Fig. 1, B-mode Lv). Because the sciatic nerve moves in an antero-posterior direction during ankle movement (Boyd et al., 2012), the ROI was manually adjusted for each frame (i.e., each second). The shear wave velocity data extracted from this ROI were averaged to obtain a representative value. Each map was visually inspected for saturated values, i.e., values that reached the maximal measurable shear wave velocity (14.1 m/s for the version of the SSI device we used). When observed, these regions of saturated values did not consistently appear on all measurements (i.e., elasticity maps) making us think that they were artifacts related to measurement rather than true regions of high stiffness. As a result of this inspection, one participant was excluded and therefore data from the remaining 9 participants were analyzed.

For the first post-conditioning cycle, the area under the shear wave velocity–ankle angle load curve, the area under the unload curve, and the normalized hysteresis area were calculated as described by Nordez et al. (2008).

As the maximum ROM varied between participants, the ankle angle was normalized to the maximal ROM. Zero percent was defined as the starting position (i.e., 40° of plantarflexion) and 100% as the end position (i.e., 80% of maximum ankle dorsiflexion). Shear wave velocity, ankle angle and EMG data were synchronised by recording a trigger from the ultrasound device (ADInstruments, Powerlab 16/35, New Zealand). Synchronised data were analyzed for the first and fifth conditioning cycles, and for the three post-conditioning cycles performed at 2°/s. Linear interpolation was used to calculate the shear wave velocity every 2° of ankle angle in order to combine it with mechanical data.

### 2.6. Statistics

The IBM SPSS software (version 20.0; IBM Corporation, New York, USA) was used for the statistics procedures. Distributions consistently passed the Kolmogorov–Smirnov normality test. All data are reported as mean ± standard deviation (SD).

The reliability of the shear wave velocity was determined across the 3 post-conditioning repetitions, at three ankle angles (20° dorsiflexion; neutral position (0°); and 20° plantarflexion) for both knee conditions during the loading phase (ankle dorsiflexion). The interclass coefficient correlation ($ICC_{[2,1]}$), standard error of measurement (SEM), and coefficient of variation (CV) were calculated (Hopkins, 2000).

To test whether the effect of ankle movements on nerve shear wave velocity was different between the two knee positions a two-way repeated-measures ANOVA was conducted on data from the first post-conditioning cycle [within-subject factors: Ankle angle with 11 levels (0–100% of total ROM with 10% increments) and Knee position with 2 levels (knee 90° and knee 180°)]. To determine whether a hysteresis occurred, a comparison between the areas under the load curve and unload curves of the shear wave velocity–ankle angle relationships was performed for both knee 180° and knee 90° using two separate paired *T*-tests.

To test the effect of repeated ankle passive movements on the sciatic nerve shear wave velocity a repeated measures ANOVA was performed for knee 180° condition only [within-subject factors: Ankle angle with 11 levels (0–100% of total ROM with 10% increments) and Cycle with 2 levels (1st and 5th cycle)].

Post-hoc analyses were performed when appropriated using Bonferroni correction method for multiple comparisons. The statistical significance was set at $p < 0.05$.

## 3. Results

The normalized RMS-EMG values remained lower than 1% of MVC for all the examined muscles (MG, LG, TA, SOL and ST) and participants allowing us to consider that the ankle dorsiflexions were performed in a passive state. A typical example of the shear wave velocity *vs* ankle angle relationship is depicted in Fig. 2. The repeatability of the nerve shear wave velocity was good at each ankle angle for both experimental conditions (i.e., knee 180° and the knee 90°). All SEM and CV values were lower than 0.34 m/s and 8.1%, respectively (Table 1). All ICC values were higher than 0.92 (Table 1).

There was a significant main effect of both ankle angle ($p < 0.0001$) and knee position ($p < 0.0001$) on nerve shear wave velocity. A significant interaction between ankle angle and knee position was also found ($p < 0.0001$) showing that although nerve shear wave velocity increased with ankle dorsiflexion for knee 180°, no changes were observed for knee 90°. Consequently, the nerve shear wave velocity was significantly higher for knee 180° than knee 90° at each ankle angle (all $p$ values $\leq 0.001$; Fig. 3). For knee 180°, the shear wave velocity was significantly higher at 70%, 80%, 90% and 100% of ankle angle relative to 0% ($p$ values $\leq 0.002$). For knee 90°, no significant changes in shear wave velocity were observed for any ankle angles compared to the 0% value ($p$ values $\geq 0.056$). The average increase of shear wave velocity was 33.7 ± 9.4% (range: 25.4–47.4% for 0% and 100% of ankle motion, respectively). For example, at 100% of ankle ROM shear wave velocity was 10.4 ± 2.4 and 5.3 ± 1.1 m/s for knee 180° and knee 90°, respectively.

When the knee was at 180°, the area under the loading curve was significantly higher ($p=0.001$) than the area under the unloading curve during the first ankle motion (Fig. 4). The normalized hysteresis area was 9.5 ± 5.2%. No significant difference ($p=0.58$) was found when the knee was at 90° (average difference: −0.3 ± 2.0%).

Although a main effect of ankle angle ($p=0.0001$) was found (similar to that reported above), neither a main effect of Cycle ($p=0.055$) nor an interaction between ankle angle and cycle ($p=0.340$) were found for the five conditioning ankle cycles.

## 4. Discussion

We investigated the shear wave velocity (an index of stiffness) of the sciatic nerve during passive ankle rotation at two knee angles. The present study shows that: *i*) SSI provides a reliable *in vivo* measurement of sciatic nerve shear wave velocity during ankle rotation; *ii)* sciatic nerve shear wave velocity increases with the ankle dorsiflexion, but this is only observed when the knee is extended at 180°; and *iii)* there was a tendency for the sciatic nerve shear wave velocity to decrease throughout the repetition of the five repeated ankle rotation motions.

A high reproducibility of SSI measurements has been previously observed in the median nerve, however only in resting conditions (Kantarci et al., 2014). Our results showed a good repeatability of the sciatic shear wave velocity measured at the proximal third of the thigh during passive and slow ankle movements (Table 1). These results highlight the potential of using shear wave elastography to non-invasively evaluate the mechanical properties of peripheral nerves at rest and during passive stretching.

The sciatic nerve stiffness increased substantially during dorsiflexion for the knee 180° condition, but no changes were observed when the knee was flexed (knee 90°) (Fig. 3). These observations are in line with previous studies, which showed that ankle dorsiflexion was associated with distal excursion of the sciatic nerve and its corresponding tibial branch (Coppieters et al., 2006). Taken together, these results show that sciatic nerve tension increases when is stretched due to specific positioning of multiple joints that the sciatic nerve crosses. This mechanical tensile response has already been shown in animal and *in vitro* studies (Silva et al., 2014; Topp and Boyd, 2006). Moreover, mechanical forces acting on peripheral nerves are thought to be transmitted well beyond the moving joint (Coppieters et al., 2006; Coppieters and Butler, 2008). Thus, the anatomical features of the sciatic nerve may explain the absence of stiffness changes during ankle rotation when the knee was flexed at 90°. The sciatic nerve (i.e., tibial and peroneal related branches) runs along the popliteal crease just behind the sagittal axis of rotation of the knee (Vloka et al., 2001). Therefore, when the knee is flexed, the structures crossing the joint may fold and slacken the nerve. Further investigations are needed to determine the effects of the cumulative nerve tensioning that may be induced by the position of the joints that it crosses.

In the present study the measurements of nerve stiffness were performed proximally, therefore at relatively large distance from

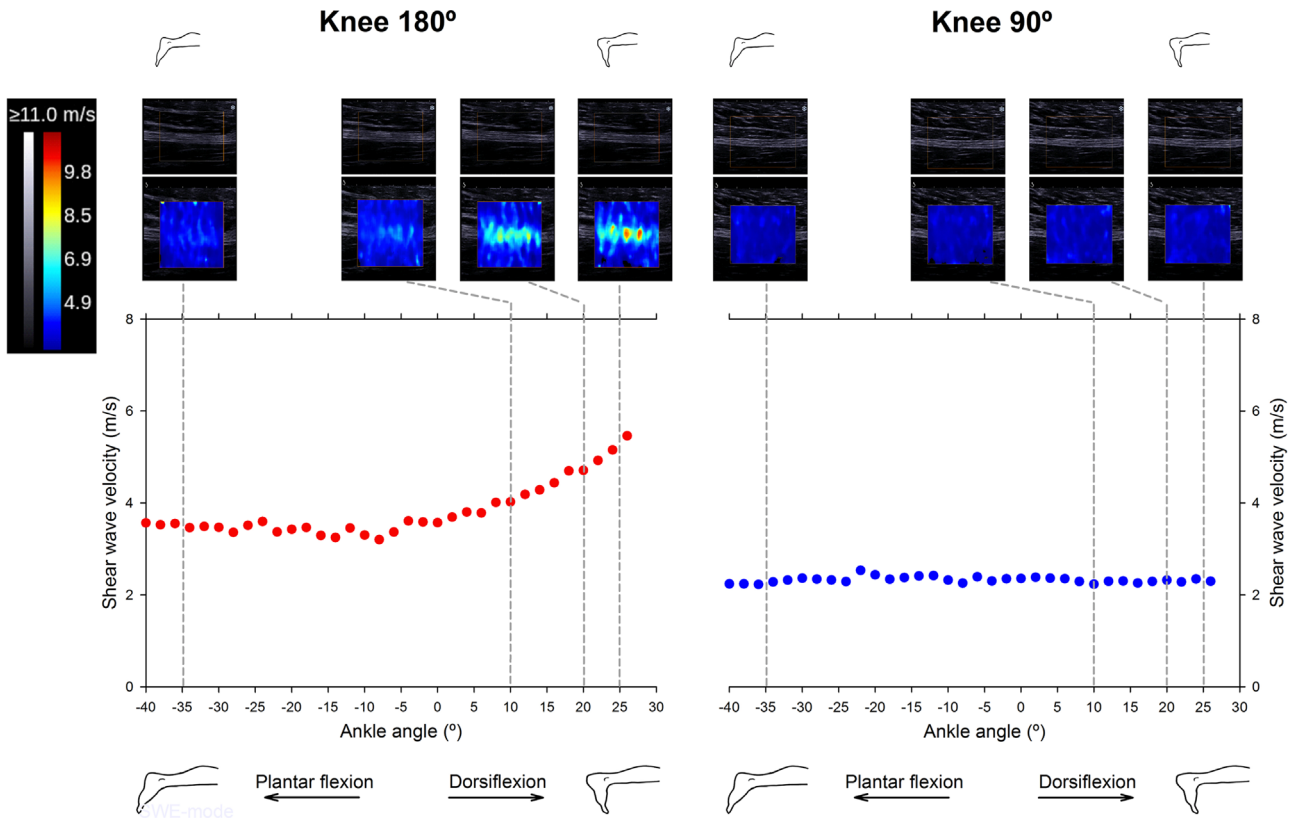


**Fig. 2.** Typical example of the nerve shear wave velocity during passive ankle dorsiflexion for both experimental conditions. Knee 180° – relationship between ankle angle and shear wave velocity with the knee fully extended. Knee 90° – relationship between ankle angle and shear wave velocity with the knee flexed. The upper panels correspond to the shear wave velocity maps obtained at −35°, 10°, 20° and 25° of ankle dorsiflexion.

**Table 1**
Repeatability of the sciatic shear wave velocity for both experimental conditions (knee 180° and knee 90°) at three ankle angles. ICC – Interclass correlation coefficient; SEM – Standard error of measurement; CV – Coefficient of variation.

| Ankle position | Knee 180° | | | Knee 90° | | |
|---|---|---|---|---|---|---|
| | ICC (95%CI) | SEM (m/s) | CV (%) | ICC (CI) | SEM (m/s) | CV (%) |
| **Plantar flexion (−20°)** | 0.97 [0.90;0.99] | 0.21 | 5.0 | 0.98 [0.93;1.00] | 0.17 | 2.9 |
| **Neutral** | 0.98 [0.93;1.00] | 0.17 | 4.0 | 0.97 [0.89;0.99] | 0.21 | 4.5 |
| **Dorsiflexion (20°)** | 0.99 [0.95;1.00] | 0.14 | 3.6 | 0.92 [0.72;0.98] | 0.34 | 8.1 |

the moved ankle joint. Nerve architecture is different between joint and non-joint areas (Phillips et al., 2004). Moreover, an *in situ* animal study showed that the nerve regions near the joints undergo greater elongation than other regions during joint movement (Phillips et al., 2004). Together, these observations suggest that the interpretation of our results should be confined to the specific location where the stiffness was measured. Further studies are needed to characterize the spatial variability of nerve stiffness behaviour during limb movements.

Our results showed that there is a tendency for the nerve shear wave velocity to decrease throughout the repetition of five ankle rotations, despite the level of statistical significance not being reached ($p=0.055$). This lack of significance is likely explained by a lack of statistical power associated to our small sample size. In a previous *in situ* animal study, cyclic sciatic nerve stretching was performed at different pre-strain levels (i.e., induced by an end-to-end anastomosis surgical approach) (Orf and Wust, 1979). The

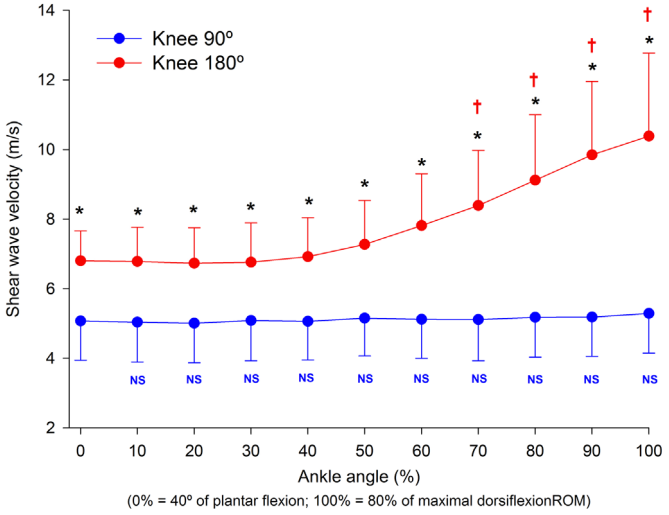


**Fig. 3.** Shear wave velocity and ankle angle relationship for both experimental conditions. 0% of ankle angle = starting test position (40° of ankle plantarflexion); 100% of ankle dorsiflexion angle = 80% of maximal dorsiflexion angle. *Shear wave velocity was significantly greater for knee 180° *vs* knee 90° across all ankle angle increments (every 10% from 0% to 100%): $p$ values ranging from 0.001 to 0.002. † For knee 180°, shear wave velocity was significantly higher at 70%, 80%, 90% and 100% relative to the 0% of ankle angle: $p$ values ranging from 0.001 to 0.002. NS For knee 90°, there were no significant differences between the 0% and all other ankle angles increments (from 10% to 100%): $p$ values higher than 0.056.

repetitive nerve stretching did not alter the stress–strain curves when the nerve was pre-strained below 8% of the initial nerve length. However, for higher pre-strained levels (i.e., 8% and 10% of the initial nerve length) a significant effect of repeated joint rotation was found. It is therefore possible that further stretch

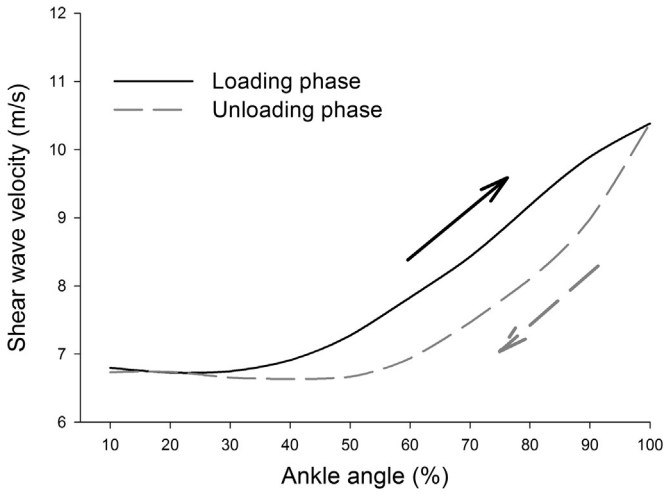


**Fig. 4.** Averaged load and unload curves (shear wave velocity of sciatic nerve) during ankle passive movement for knee extended (knee 180°) condition. 0% corresponds to 40° of plantarflexion and 100% to 80% of maximum dorsiflexion. The normalized hysteresis area was calculated as Nordez et al. (2008). Knee 90° was not represented due to a lack of changes in load and unload curves.

applied to the sciatic nerve (e.g., by changing the hip position or by stretching the ankle to $>80\%$ of maximal ROM) might have induced a significant effect of loading cycles on nerve stiffness.

The area under the loading curve was significantly higher than the area under the unloading curve when the knee was extended (knee 180°). This indicates the presence of a hysteresis, which was expected as the nerve is a viscoelastic tissue (Topp and Boyd, 2006). Although this result is in accordance with previous *in situ* and *in vitro* studies (Chen et al., 2010; Ju et al., 2006; Rickett et al., 2011), it was not demonstrated *in vivo* so far. The absence of hysteresis observed in knee 90° is likely explained by the lack of tensioning of proximal region of the sciatic nerve during ankle dorsiflexion (i.e., no loading).

There are a couple of methodological issues that have to be discussed and addressed in the future works. First, a spatial variability of shear wave velocity was observed (e.g. nerve maps at 20° and 25° on Fig. 2 – Knee 180°). Similar observations were reported from stretched muscles (Freitas et al., 2015; Le Sant et al., 2015) or tendons (Hug et al., 2013). This variability is likely related to the variability in both architecture and composition of these tissues. To limit the influence of this variability on our results, the shear wave velocity values were averaged over a representative ROI that remained the same throughout the entire ankle ROM. Second, as observed in the B-mode image of the Fig. 1 (transverse view), the sciatic nerve has an asymmetric architecture. It is unknown whether this asymmetry is related to a spatial variability of its mechanical properties. Because the spatial resolution of the SSI technique is limited to $1 \times 1$ mm$^2$ and because the nerve is a thin structure, this variability could not be assessed in our study.

Finally, there is evidence that nerve mechanical properties influences both the efficiency of nerve functions and the protection of the nerve fascicles (Topp and Boyd, 2006). For example, the amplitude of the compound action potential decreases linearly as the nerve strain increases (Li and Shi, 2007; Rickett et al., 2011; Wall et al., 1992). Furthermore, it is particularly important to consider that changes in nerve morphology can be triggered by pathophysiological processes [e.g., modifications in collagen content, fibril diameter, cross sectional area and intraneural pressure (Di Pasquale et al., 2015; Goedee et al., 2013; Topp and Boyd, 2006)], and therefore are likely to affect nerve tension and stiffness during movements. Consequently, the non-invasive characterization of the nerve mechanical properties proposed in the present study may be clinically relevant to allow the clinician to: 1) diagnose a pathological nerve; 2) design appropriate treatment techniques based on manual therapy (i.e., neural tension *vs* neural excursion); and 3) have real-time feedback of the intervention minimizing the physical stress that is induced on the injured nerve.

## 5. Conclusion

In summary, we showed that the shear wave velocity (an index of stiffness) of the sciatic nerve can be experimentally assessed during slow passive ankle rotation using SSI. In addition, this study demonstrates that the shear wave velocity of the sciatic nerve is influenced by the knee position regardless of the ankle joint, suggesting a cumulative tensioning effect of the nerve induced by the position of multiple joints. An increase in nerve shear wave velocity during ankle dorsiflexion was observed only when the knee was extended. Finally, the nerve shear wave velocity was not affected by five stretching repetitions. This is the first study that non-invasively assesses nerve stiffness during a joint movement. The notable dependence of the sciatic tension on nerve mechanics induced by the joints' positioning, which the nerve crosses, should be considered during clinical assessment and interventions (e.g., to minimize physical stress on an injured nerve). Further investigations are therefore required to characterize the peripheral nerves mechanics during joints movement, in both healthy and clinical populations (e.g., specific peripheral neuropathies).

## Conflict of interest

The authors have no conflict of interest.

## Acknowledgements

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## References

Beekman, R., Visser, L.H., 2004. High-resolution sonography of the peripheral nervous system – a review of the literature. Eur. J. Neurol. 11, 305–314.
Bercoff, J., Tanter, M., Fink, M., 2004. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. IEEE Trans. Ultrason. Ferroelectr. Freq. Control 51, 396–409.
Boyd, B.S., Dilley, A., 2014. Altered tibial nerve biomechanics in patients with diabetes mellitus. Muscle Nerve 50, 216–223.
Boyd, B.S., Gray, A.T., Dilley, A., Wanek, L., Topp, K.S., 2012. The pattern of tibial nerve excursion with active ankle dorsiflexion is different in older people with diabetes mellitus. Clin. Biomech. 27, 967–971.
Boyd, B.S., Topp, K.S., Coppieters, M.W., 2013. Impact of movement sequencing on sciatic and tibial nerve strain and excursion during the straight leg raise test in embalmed cadavers. J. Orthop. Sports Phys. Ther. 43, 398–403.
Bruhn, J., Moayeri, N., Groen, G.J., A, V.A.N.V., Gielen, M.J., Scheffer, G.J., GJ,, V.A.N.G., 2009. Soft tissue landmark for ultrasound identification of the sciatic nerve in the infragluteal region: the tendon of the long head of the biceps femoris muscle. Acta Anaesthesiol. Scand. 53, 921–925.
Bruhn, J., Van Geffen, G.J., Gielen, M.J., Scheffer, G.J., 2008. Visualization of the course of the sciatic nerve in adult volunteers by ultrasonography. Acta Anaesthesiol. Scand. 52, 1298–1302.
Brum, J., Bernal, M., Gennisson, J.L., Tanter, M., 2014. In vivo evaluation of the elastic anisotropy of the human Achilles tendon using shear wave dispersion analysis. Phys. Med. Biol. 59, 505–523.
Bueno, F.R., Shah, S.B., 2008. Implications of tensile loading for the tissue engineering of nerves. Tissue Eng. Part B Rev. 14, 219–233.
Butler, D.S., 2000. The Sensitive Nervous System. Noigroup Publications, Adelaide, Australia.
Chen, R.J., Lin, C.C., Ju, M.S., 2010. In situ transverse elasticity and blood perfusion change of sciatic nerves in normal and diabetic rats. Clin. Biomech. 25, 409–414.

Coppieters, M.W., Alshami, A.M., Babri, A.S., Souvlis, T., Kippers, V., Hodges, P.W., 2006. Strain and excursion of the sciatic, tibial, and plantar nerves during a modified straight leg raising test. J. Orthop. Res. 24, 1883–1889.
Coppieters, M.W., Butler, D.S., 2008. Do'sliders' slide and'tensioners' tension? An analysis of neurodynamic techniques and considerations regarding their application. Man. Ther. 13, 213–221.
DeWall, R.J., Slane, L.C., Lee, K.S., Thelen, D.G., 2014. Spatial variations in Achilles tendon shear wave speed. J. Biomech. 47, 2685–2692.
Di Pasquale, A., Morino, S., Loreti, S., Bucci, E., Vanacore, N., Antonini, G., 2015. Peripheral nerve ultrasound changes in CIDP and correlations with nerve conduction velocity. Neurology 84, 803–809.
Dilley, A., Greening, J., Lynn, B., Leary, R., Morris, V., 2001. The use of cross-correlation analysis between high-frequency ultrasound images to measure longitudinal median nerve movement. Ultrasound Med. Biol. 27, 1211–1218.
Eby, S.F., Song, P., Chen, S., Chen, Q., Greenleaf, J.F., An, K.N., 2013. Validation of shear wave elastography in skeletal muscle. J. Biomech. 46, 2381–2387.
Ellis, R.F., Hing, W.A., McNair, P.J., 2012. Comparison of longitudinal sciatic nerve movement with different mobilization exercises: an in vivo study utilizing ultrasound imaging. J. Orthop. Sports Phys. Ther. 42, 667–675.
Freitas, S.R., Andrade, R.J., Larcoupaille, L., Mil-homens, P., Nordez, A., 2015. Muscle and joint responses during and after static stretching performed at different intensities. Eur. J. Appl. Physiol. 115, 1263–1272.
Goedee, H.S., Brekelmans, G.J., van Asseldonk, J.T., Beekman, R., Mess, W.H., Visser, L.H., 2013. High resolution sonography in the evaluation of the peripheral nervous system in polyneuropathy–a review of the literature. Eur. J. Neurol. 20, 1342–1351.
Hermens, H.J., Freriks, B., Disselhorst-Klug, C., Rau, G., 2000. Development of recommendations for SEMG sensors and sensor placement procedures. J. Electromyogr. Kinesiol. 10, 361–374.
Hopkins, W.G., 2000. Measures of reliability in sports medicine and science. Sports Med. 30, 1–15.
Hough, A.D., Moore, A.P., Jones, M.P., 2007. Reduced longitudinal excursion of the median nerve in carpal tunnel syndrome. Arch. Phys. Med. Rehabil. 88, 569–576.
Hug, F., Lacourpaille, L., Maisetti, O., Nordez, A., 2013. Slack length of gastrocnemius medialis and Achilles tendon occurs at different ankle angles. J. Biomech. 46, 2534–2538.
Jou, I.M., Lai, K.A., Shen, C.L., Yamano, Y., 2000. Changes in conduction, blood flow, histology, and neurological status following acute nerve-stretch injury induced by femoral lengthening. J. Orthop. Res. 18, 149–155.
Ju, M.S., Lin, C.C., Fan, J.L., Chen, R.J., 2006. Transverse elasticity and blood perfusion of sciatic nerves under in situ circular compression. J. Biomech. 39, 97–102.
Kantarci, F., Ustabasioglu, F.E., Delil, S., Olgun, D.C., Korkmazer, B., Dikici, A.S., Tutar, O., Nalbantoglu, M., Uzun, N., Mihmanli, I., 2014. Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. Eur. Radiol. 24, 434–440.
Karmakar, M.K., Kwok, W.H., Ho, A.M., Tsang, K., Chui, P.T., Gin, T., 2007. Ultrasound-guided sciatic nerve block: description of a new approach at the subgluteal space. Br. J. Anaesth. 98, 390–395.
Koo, T.K., Guo, J.Y., Cohen, J.H., Parker, K.J., 2013. Relationship between shear elastic modulus and passive muscle force: an ex-vivo study. J. Biomech. 46, 2053–2059.
Kwan, M.K., Wall, E.J., Massie, J., Garfin, S.R., 1992. Strain, stress and stretch of peripheral nerve. Rabbit experiments in vitro and in vivo. Acta Orthop. Scand. 63, 267–272.
Le Sant, G., Ates, F., Brasseur, J.L., Nordez, A., 2015. Elastography study of hamstring behaviors during passive stretching. PloS One 10, e0139272.
Li, J., Shi, R., 2007. Stretch-induced nerve conduction deficits in guinea pig ex vivo nerve. J. Biomech. 40, 569–578.
Maganaris, C.N., 2003. Tendon conditioning: artefact or property? Proc. Biol. Sci. 270 (Suppl 1), S39–S42.
Maisetti, O., Hug, F., Bouillard, K., Nordez, A., 2012. Characterization of passive elastic properties of the human medial gastrocnemius muscle belly using supersonic shear imaging. J. Biomech. 45, 978–984.
McNair, P.J., Hewson, D.J., Dombroski, E., Stanley, S.N., 2002. Stiffness and passive peak force changes at the ankle joint: the effect of different joint angular velocities. Clin. Biomech. 17, 536–540.
Millesi, H., Zoch, G., Reihsner, R., 1995. Mechanical properties of peripheral nerves. Clin. Orthop. Relat. Res., 76–83.
Nordez, A., McNair, P., Casari, P., Cornu, C., 2008. Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. Int. J. Sports Med. 29, 414–418.
Orf, G., Wust, R., 1979. Mechanical loading of peripheral nerves during remobilisation of the affected member after end-to-end anastomosis. Acta Neurochir. 49, 103–121.
Phillips, J.B., Smit, X., De Zoysa, N., Afoke, A., Brown, R.A., 2004. Peripheral nerves in the rat exhibit localized heterogeneity of tensile properties during limb movement. J. Physiol. 557, 879–887.
Rickett, T., Connell, S., Bastijanic, J., Hegde, S., Shi, R., 2011. Functional and mechanical evaluation of nerve stretch injury. J. Med. Syst. 35, 787–793.
Silva, A., Manso, A., Andrade, R., Domingues, V., Brandao, M.P., Silva, A.G., 2014. Quantitative in vivo longitudinal nerve excursion and strain in response to joint movement: a systematic literature review. Clin. Biomech. 29, 839–847.
Slane, L.C., DeWall, R., Martin, J., Lee, K., Thelen, D.G., 2015. Middle-aged adults exhibit altered spatial variations in Achilles tendon wave speed. Physiol. Meas. 36, 1485–1496.
Topp, K.S., Boyd, B.S., 2006. Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. Phys. Ther. 86, 92–109.
Vloka, J.D., Hadzic, A., April, E., Thys, D.M., 2001. The division of the sciatic nerve in the popliteal fossa: anatomical implications for popliteal nerve blockade. Anesth. Analg. 92, 215–217.
Wall, E.J., Massie, J.B., Kwan, M.K., Rydevik, B.L., Myers, R.R., Garfin, S.R., 1992. Experimental stretch neuropathy. Changes in nerve conduction under tension. J. Bone Joint Surg. Br. 74, 126–129.

# STUDY 3

## Sciatic nerve stiffness plays a key role in the limitation of maximal ankle dorsiflexion range of motion

**Andrade RJ**, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Gross R, McNair PJ & Nordez A. (Submitted)

SCIATIC NERVE STIFFNESS PLAYS A KEY ROLE IN THE LIMITATION OF MAXIMAL ANKLE DORSIFLEXION RANGE OF MOTION

*Medicine & Science in Sports & Exercise*

# ABSTRACT

It is a long held belief that maximal joint range of motion (ROM) is restricted by muscle tension. However, it exists indirect evidence that this assumption may not hold true for some joint configurations where non-muscular structures, such as the peripheral nerves, are stretched and as consequence may limit the maximal ROM. Direct evidences are lacking. This study aimed to determine whether static stretching that aims to load the sciatic nerve without stretch within plantar flexors is effective to: i) alter nerve stiffness; and ii) increase the ankle's maximal ROM.

Passive maximal ankle ROM in dorsiflexion was assessed with the hip flexed at 90° (HIP-flexed) or neutral (HIP-neutral, 0°). Sciatic nerve stiffness was estimated using shear wave elastography.

Sciatic nerve stretching induced both a 13.3 ± 7.9 % ($P < 0.001$) decrease in the nerve stiffness and a 6.4 ° ± 2.6° increase (+ 88.9 %; $P < 0.001$) in the maximal ROM in dorsiflexion assessed with the hip flexed (i.e., the position that maximally stretches the nerve). In addition, the decrease in sciatic nerve stiffness was significantly correlated to the change in maximal ROM in dorsiflexion ($r = -0.571$, $P = 0.026$). These effects occurred in the absence of any change in *gastrocnemius medialis* and *biceps femoris long head* stiffness, and ankle passive torque.

### *Contribution to this doctoral research:*

- These results demonstrate that maximal ankle dorsiflexion ROM can be considerably increased by stretching the sciatic nerve, without altering the muscle stiffness and ankle torque;

- Long-sitting position (HIP-flexed) can be used as a non-muscular stretching technique to target adaptations in the sciatic and maximal dorsiflexion ROM without load *triceps surae*.

***

***Keywords:*** *Sciatic stiffness; Peripheral nerves; Nerve stretch; Ankle; Supersonic shear imaging*

# Sciatic nerve stiffness plays a key role in the limitation of maximal ankle dorsiflexion range of motion

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# INTRODUCTION

The maximal range of motion (ROM) of a joint is an important functional parameter to estimate the maximal muscle length or muscle extensibility (15) that is widely used in clinical practice, sport training and research. It is a long-held belief that maximal ankle ROM in dorsiflexion is restricted by the tension within the plantar flexor muscles, i.e. either the maximal tension that can be reached (i.e., "mechanical theory") or the perception of this tension (i.e., "sensory theory") (36). However, although during ankle rotations many other non-muscular tissues (e.g. peripheral nerves and lower limb *fasciae*) experience large changes in their length and tension (1, 3, 8, 9), the influence of these non-muscular tissues on the maximal ROM at a joint remains largely unexplored.

Two previous studies observed a remarkable decrease in the maximal ankle ROM in dorsiflexion when the hip was flexed from the neutral position to 90° (2, 26). This change occurred in the absence of any changes in the *gastrocnemius medialis* (GM) stiffness and ankle torque (2). Because there is no muscle-tendon unit crossing both the hip and ankle joints, these results suggested that non-muscular structures may limit the maximal ankle ROM in dorsiflexion (27). A possible candidate to explain this effect is the sciatic nerve that extends from the spine through branches to the foot, and like most other connective tissues, exhibits visco-elastic behavior (12). If so, a sciatic nerve stretch may decrease the stiffness of the neural tissue and improve the maximal ankle dorsiflexion ROM.

To test this hypothesis non-invasively, it is necessary to assess the sciatic nerve stiffness during passive ankle rotations. An understanding of the biomechanical properties of peripheral nerves would have strong clinical importance, in particular to the management of nerves in the context of entrapment neuropathy or chronic neuropathies where both nerve stiffness and ROM are known to be affected (11, 22, 31). Ultrasound shear wave elastography can be used to assess *in vivo* the shear wave velocity within soft tissues (16, 21), this parameter being directly related to the shear modulus of the tissue (4). It therefore provides an accurate characterization of muscle stiffness (13). Recently, it was showed that this technique can be used to reliably measure the shear wave velocity within the sciatic nerve during passive ankle rotations (3).

This study was designed to appreciate the role of the sciatic nerve stiffness in the maximal ankle ROM in dorsiflexion. An experimental protocol was conducted in humans to determine whether a total of 6-min static stretch that aims to load the sciatic nerve without stretch within plantar flexors is effective to: i) alter nerve stiffness; and ii) increase the ankle's maximal ROM. The sciatic stiffness was measured, before and after the 6-min static stretch, while the nerve was progressively stretched during ankle dorsiflexion. We hypothesized that: i) it is possible to selectively decrease the sciatic nerve stiffness without altering the muscle stiffness; and ii) the specific sciatic stretch would induce an increase in maximal ankle ROM in dorsiflexion.

## MATERIALS AND METHODS

### Participants

Fifteen healthy volunteers participated in this study (13 males and 2 females; age: 22 ± 3 years, height: 175 ± 7 cm, weight: 66 ± 7 kg). Participants had no history of significant trauma or surgery to the spine, hip or hamstring region, knee and ankle joints, or symptoms consistent with sciatic or tibial nerve pathology. The straight leg raising and slump tests (34) had to be negative in all participants, to prevent the inclusion of subjects with pathology. Participants were not engaged in any flexibility training, and were asked to avoid intense exercise 48 h prior to the testing sessions. They were informed about methods used in this study before providing written informed consent. The Institutional Ethics Committee approved the study, and all procedures conformed to the Declaration of Helsinki (last modified in 2013).

### Ergometer

An isokinetic dynamometer (Con-Trex MJ; CMV AG, Dubendorf, Switzerland) was used to perform passive ankle rotations and to measure the ankle torque. All measurements were performed on the right ankle joint. The lateral malleolus was used to estimate the center of rotation of the ankle, and was aligned with the axis of the ergometer. The neutral position of the ankle (0º) was defined as an angle of 90º between the footplate and the shank. The foot was firmly strapped such that the potential heel displacement from the dynamometer platform was minimized during the ankle rotations. Ankle angle and torque data were collected at 100 Hz with an analogue/digital

converter (ADInstruments, Colorado Springs, CO, USA). A standard goniometer (MSD, Londerzeel, Belgium) was used to set the hip position (flexed at 90° or neutral - i.e. 180°) and knee (fully extended, i.e. 180°) positions. With the exception of the hip joint, the participant's positioning (i.e. knee and ankle joints) remained unchanged throughout all the testing.

*Testing position*

Participants were tested for maximal ankle ROM at two hip positions in a random order: HIP-neutral and HIP-flexed. For the HIP-neutral position, participants laid prone (figure 1C) on the dynamometer. The contralateral limb remained straight in a relaxed position. The knee was fully extended and strapped securely in this position such that no knee motion occurred during ankle rotations. For the HIP-flexed position (Figures 1D), participants were seated with the hip flexed at 90º and the knee fully extended. The contralateral limb was flexed to 90° (hip and knee) and remained relaxed. The chest and the waist were strapped to minimize trunk motion during ankle rotations.

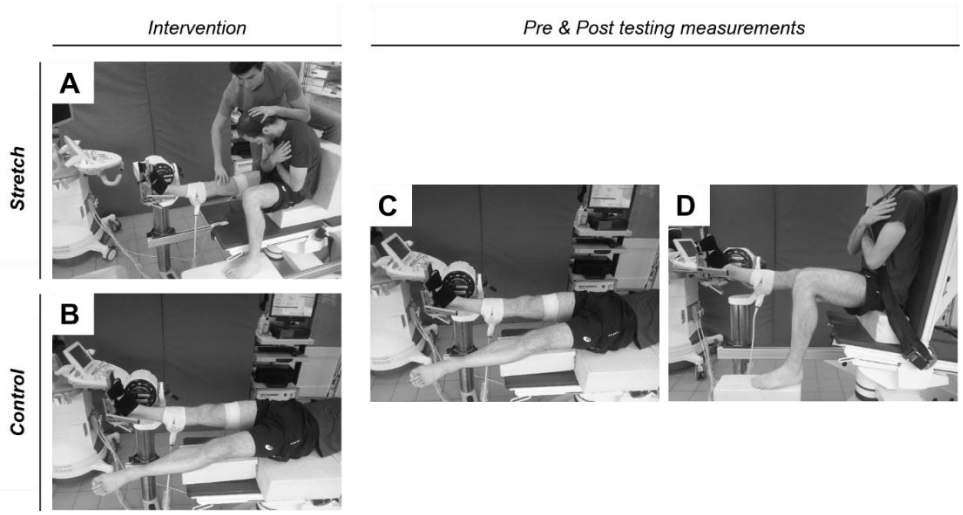


Figure 1. Testing positions used for maximal ankle ROM in dorsiflexion and elastography measurements. **A.** Stretch position used to stretch the sciatic nerve. Hip and spine were flexed to maximum tolerable stretch limit, and ankle angle was set as neutral; **B.** HIP-neutral position used as intervention in Control session (ankle was positioned in neutral); **C.** HIP-neutral position used for maximal ankle ROM, elastography (sciatic nerve, *biceps femoris* and *gasctrocnemius medialis*) and torque measurements before and after the intervention; **D.** HIP-flexed position used for maximal ankle ROM in dorsiflexion assessment.

**Shear Wave Elastography**

All the assessments were performed in HIP-neutral (Figure 1C). The reason being that sciatic nerve elastography measurements were not reliable in HIP-flexed position (see details in discussion). We assessed the shear wave velocity of the sciatic nerve, the *medial gastrocnemius* (GM) and the long head of *biceps femoris* (BF) muscles. To this end, an Aixplorer ultrasound scanner (version 6.1; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer (4-15 MHz for GM muscle or 2-10 MHz for the sciatic nerve and the BF muscle, Super Linear, Aix-en- Provence, France) was used in shear wave elastography mode. The region of interest (ROI) was first identified in B-mode images. For the sciatic nerve, the transducer was positioned parallel to the nerve fibers on the proximal third of the thigh (3). The GM was divided in three equal regions calculated from the distance between the distal and the proximal myotendinous junctions, and measurements were performed in the intermediate region as described in by Le Sant, et al. (2017) (24). Finally, the BF muscle was measured at a location in close proximity to where the sciatic nerve signals were collected. The hamstring muscle was examined to determine: i) if it is possible load the sciatic nerve during ankle dorsiflexion without affecting the muscle's stiffness; and ii) whether the nerve stretching intervention could change the nerve stiffness without changing the BF stiffness.

The following shear wave elastography acquisition parameters were set: penetration mode, 100% opacity, no temporal smoothing (persistence=OFF), and intermediate spatial smoothing of 5/9. All the measurements were performed by an experienced ultrasonographer. The transducers were secured to the thigh (sciatic nerve and BF) or the lower leg (GM) with custom-made casts and additionally strapped to the skin to be certain that elastography measurements were performed exactly at the same location before and after the intervention. Both procedures were performed with minimal tissue compression.

An analog trigger signal originating from the ultrasound scanner at each elastography measurement was recorded to ensure a perfect synchronization between shear wave data, ankle angle, ankle torque and surface electromyography (EMG). The maps of the shear modulus were obtained at 1 sample/s and with a spatial resolution of 1 × 1 mm.

**Electromyography (EMG)**

To be assured that the lower leg muscles remained inactive during the measurements, myoelectrical activity was assessed using surface EMG. A pair of surface electrodes (Kendall 100 Series Foam Electrodes, Covidien, Massachusetts, USA) was placed over the *gastrocnemius lateralis* (GL), *tibialis anterior* (TA), *soleus* (SOL) and *semitendinosus* (ST) muscles at the location recommended by the Surface EMG for Non-Invasive Assessment of Muscles guidelines (19). GL and ST were chosen instead of GM and BF because of interference with the locations used for elastography assessments. EMG data were acquired simultaneously with the mechanical data (ADInstruments, Colorado Springs, CO, USA) at a sampling rate of 1 kHz. The EMG signals were amplified (× 1000), and band-pass filtered (8-380 Hz).

**Experimental protocol**

Each participant performed first one familiarization session. In this session, participants were familiarized with the testing set-up and maximal ankle ROM in dorsiflexion assessments and stretching protocol 24-h prior to their first testing session. Then, participants performed two main experimental testing sessions in a randomized order (established by the toss of a coin) designated 'Stretch' and 'Control' sessions. These sessions were separated by at least 24-h (mean: 172 hours) and were conducted at the same time of the day. In each testing session, all variables were measured before (pre-testing) and immediately after (post-testing) the stretching or control intervention.

At the beginning of each experimental session, five conditioning ankle rotations at 5°/s were performed in the HIP-neutral position between 40° of plantar flexion and 15° of dorsiflexion (28). The first session was divided in 4 steps. First, the maximal ROM in dorsiflexion was assessed in both HIP-neutral (Figure 1C) and HIP-flexed (Figure 1D) positions (randomized order). Two trials separated by 1-min rest were performed for each testing position. Participants were asked to completely relax with their eyes closed while the ankle was passively rotated at 2°/s from 40° plantar flexion toward maximal dorsiflexion. When the participants reached their maximum tolerable stretch limit, defined as the onset of pain in the posterior region of the leg (2), they pressed a button that immediately released the footplate, and hence the associated stretch on the soft tissue

structures. In both positions, the greatest maximal ankle ROM in dorsiflexion was attained during these trials retained for statistical analysis procedures.

Secondly, over three repetitions, elastography measurements were performed on GM, BF and sciatic nerve (randomized order) while the ankle was passively rotated (2°/s) from 40° of plantar flexion to the maximal ROM in dorsiflexion previously determined in HIP-neutral (see Figure 1C). Next, participants underwent a total of 6-min (2× 180 s with a 30 s rest interval) rest (Control session) or nerve stretching (Stretch session) immediately after the pre-testing. Specifically, the nerve stretching was performed by an experienced physiotherapist who passively flexed the hip from the HIP-neutral (i.e. towards a long sitting position), followed by lumbar, thoracic and cervical flexion to the end of range of motion, as dictated by the participant (Figure 1A). During this manoeuver, the ankle angle was maintained in neutral position (0°) to minimize the stretch of the plantar flexor muscles. This neural stretch technique (5, 8) was performed at the maximal tolerable hip flexion ROM, and used to target the stretch of the sciatic nerve tract with minimal tension within the plantar flexors. The knee of the tested lower limb remained in full extension, while the contralateral lower limb was flexed at 90° throughout the procedure. During the Control session, participants remained relaxed for 6-min in the HIP-neutral position, with the ankle of the tested limb positioned at 0° (Figure 1B). The contralateral knee was positioned straight in a relaxed position. After 6 minutes, testing identical to the pre-intervention measurements were performed immediately after the Stretch or Control interventions.

Two maximal effort isometric contractions of plantar flexors, and dorsiflexors were performed with the ankle in a neutral position with the participants in supine; while knee flexors were tested with the knee fully extended in the same lying position. There was a 1-min rest interval between each maximal effort trial. These MVC contractions were used to normalize the EMG amplitude.

**Data analysis**

All data remained coded so that the analyses could be undertaken in a blinded manner. All data were processed using MATLAB scripts (The MathWorks Inc., Natick, USA). Angle and torque signals were low-pass filtered with a second-order Butterworth filter.The root mean square of the EMG signal (RMS EMG) was calculated over 300-ms windows throughout the testing procedures.

Artifacts originating from the supersonic push beam (1 sample/s) were discarded from EMG. The RMS EMG values were normalized to that recorded during maximal voluntary isometric contractions.

Both muscle and nerve stiffness estimation were based on shear wave velocity (Vs) quantification. The Vs is directly linked to the shear modulus ($\mu$) (4), $\mu = \rho Vs^2$, where $\rho$ is the estimated density of soft tissues (1000 kg/m$^3$). Videos were exported from the Aixplorer's scanner on 'mp4' format, and then sequenced in 'jpeg' images. The image processing converted each pixel of the color map into a shear wave velocity value based on the recorded color scale (scale = 0-18.3 m/s). A region of interest (ROI) was first defined on the first map as the largest muscle or nerve area. Then this ROI was manually tracked every second during the recording performed for each ankle rotation in dorsiflexion. Each ROI was then inspected for artifacts (saturation or void areas). If artifacts were present in any of the images to be analyzed within a recording, the ROI was reduced in size to exclude the area of artifact from all images within that recording (3). The shear wave velocity data extracted from this ROI were averaged to obtain a representative value.

Since shear wave dispersion analysis has shown that shear wave propagation is guided in Achilles tendon, the tendon thickness to influence the group shear wave velocity that can not be directly related to the shear modulus (6, 18). Although sciatic nerve is less stiff than Achilles tendon, and thus the guided shear wave propagation is less problematic (3, 10), no study has applied this shear wave dispersion analysis to peripheral nerves. Therefore, our results are reported as shear wave velocity (3). In addition, the mean sciatic nerve thickness was calculated using the B-mode ultrasound images to ensure that pre- to post-intervention changes in shear wave velocity would not have been influenced by nerve thickness adaptations to stretching. Specifically, the nerve thickness was calculated from the distance between sciatic nerve boundaries on the locations of shear wave velocity measurements.

The sciatic nerve, GM and BF shear wave velocity, ankle torque and RMS EMG were calculated every 2°, from 40° of plantar flexion to the maximal ankle ROM in dorsiflexion. Then, to standardise range of motion to a percentage of maximum, a linear interpolation method was used to calculate the sciatic, GM and BF shear wave velocity, and ankle torque every 5% of ankle ROM. For each participant, the maximum ankle ROM in dorsiflexion attained in pre-testing (Control or Stretch sessions) was used to examine the effects of stretching. This point corresponds to 100% of the pre-testing ankle ROM in dorsiflexion. At this angle, sciatic nerve, GM and BF

shear wave velocity, ankle torque and RMS EMG values were determined using a linear interpolation method, and then used for statistical analysis purposes.

### Statistics

The IBM SPSS software (version 20.0; IBM Corporation, New York, USA) was used for the statistics procedures. Distributions consistently passed the Shapiro-Wilk normality test. All data are thus reported as mean ± standard deviation (SD).

Two-way ANOVA with repeated measures [session (Stretch, Control) × time (pre, post)] was performed to identify changes in maximal ROM in dorsiflexion after the intervention in both testing positions (HIP-neutral and HIP-flexed). Nine two-way ANOVAs with repeated measures [session (Stretch, Control) × time (pre, post)] were performed to examine the effect of the intervention on the sciatic nerve and muscles shear wave velocity (GM, BF), ankle torque, sciatic nerve thickness, and RMS-EMG of GL, SOL, TA and ST. These analyses were performed at the same ankle angle, i.e. 100% of the pre-testing ankle ROM in dorsiflexion. The partial eta square ($_p\eta^2$) values were reported as a measure of the effect size of the ANOVA's findings. Small, medium and large effects were considered for $_p\eta^2 = 0.01$, $_p\eta^2 = 0.06$ and $_p\eta^2 \geq 0.14$, respectively (7). Post hoc analyses subsequent to the ANOVAs were performed when appropriated using the Bonferroni correction for multiple comparisons. The statistical significance was set at $P < 0.05$.

## RESULTS

The effects of nerve stretching on ankle torque and sciatic nerve, GM and BF shear wave velocity are summarized in Table 1 and Figure 2.

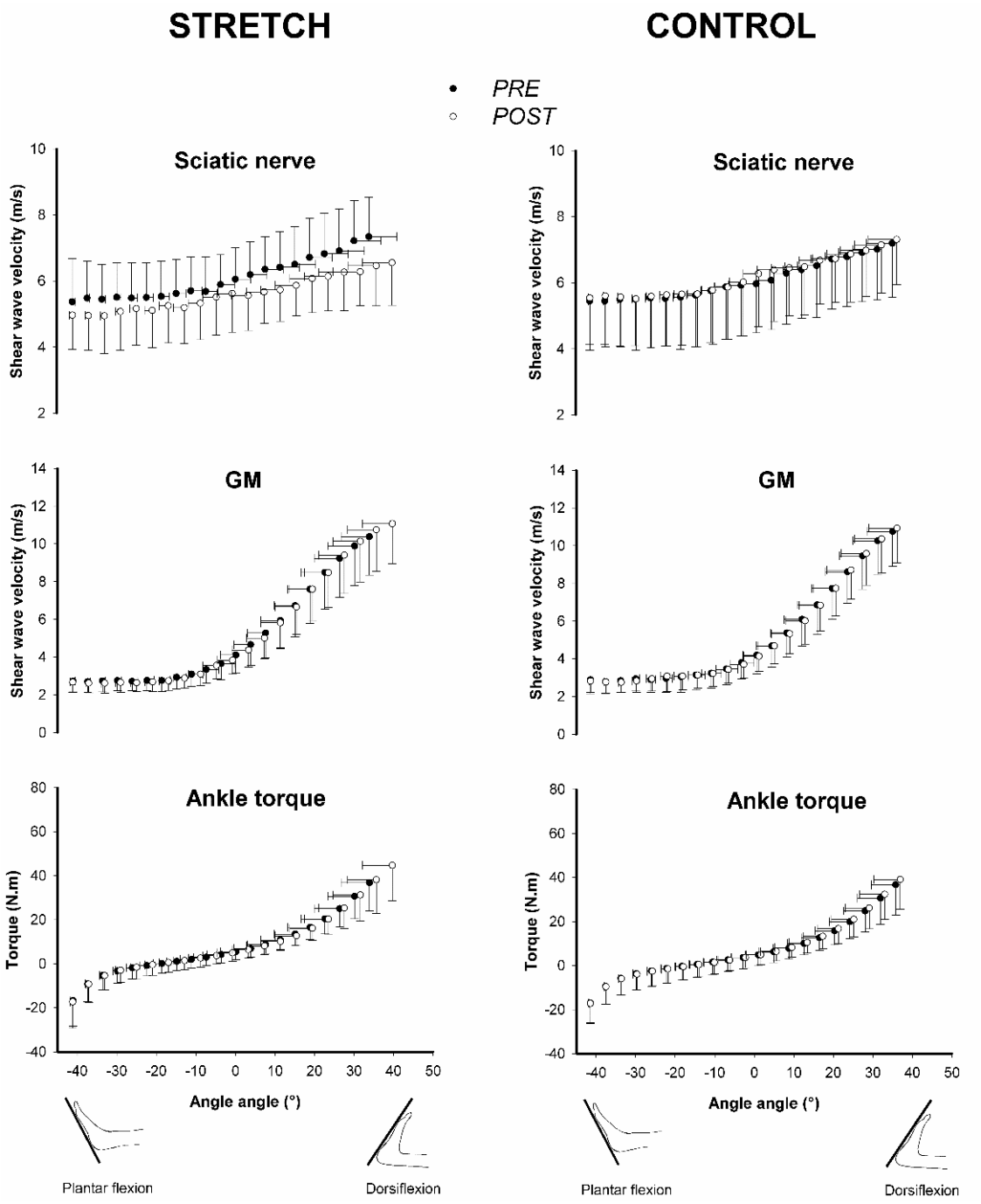


Figure 2. Relationships between the ankle angle and the sciatic nerve and *gastrocnemius medialis* (GM) shear wave velocity; and ankle torque. Measurements were performed before and immediately after the intervention in both Stretch and Control sessions. All assessments were performed in HIP-neutral position. The ankle rotation started at 40° of plantar flexion.

Table 1. Ankle passive torque, sciatic nerve, *gastrocnemius medialis* (GM) and *biceps femoris* long head (BF) shear wave velocity, and sciatic nerve thickness at the maximal ankle dorsiflexion ROM, before and after the Stretch and Control interventions.

| | Torque (N.m) | | Sciatic nerve SWV (m/s)[#] | | | GM SWV (m/s) | | BF SWV (m/s) | | Sciatic nerve thickness (cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Angle angle** | **Before** | **After** | **Before** | **After** | **P-value** | **Before** | **After** | **Before** | **After** | **Before** | **After** |
| | | | | | | **Stretch[#]** | | | | | |
| **100%[#]** (32.9° ± 7.3°) | 35.3± 12.7 | 33.4 ± 13.7 | 7.3 ± 0.3 | 6.3 ± 0.3 | 0.00001 | 18.1 ± 3.5 | 18.1 ± 3.6 | 5.1 ± 1.0 | 5.1 ± 0.8 | 0.5 ± 0.1 | 0.5 ± 0.1 |
| | | | | | | **Control** | | | | | |
| **100%** (34.0° ± 6.3°) | 34.8 ± 13.3 | 35.5 ± 13.5 | 7.3 ± 0.3 | 7.4 ± 0.4 | 0.486 | 19.1 ± 2.2 | 19.0 ± 2.2 | 5.4 ± 1.2 | 5.4 ± 1.1 | 0.5 ± 0.1 | 0.5 ± 0.1 |

Values are presented as mean + SD; Session × time interaction was found for the sciatic nerve SWV ($^{\#}p < 0.0001$) at 100% of maximal ankle ROM in dorsiflexion. *Post hoc* p-values for the before to after intervention changes in sciatic nerve shear-wave velocity (SWV) are presented for both Stretch and Control sessions. No significant session × time interactions were observed for ankle torque, GM and BF shear wave velocity, and sciatic nerve thickness. GM: *gastrocnemius medialis*; BF: *biceps femoris*; SWV: shear wave velocity; ROM: range of motion.

*Muscle shear wave velocity*

There was neither a significant session × time interaction (GM: P = 0.660; BF: P = 0.855) nor a significant main effects of time (GM: P = 0.742; BF: P = 0.696) and session (GM: P = 0.317; BF: P = 0.2) on GM and BF shear wave velocity measured at 100% of the pre-testing ankle ROM in dorsiflexion.

*Sciatic nerve shear wave velocity*

A significant session × time interaction was observed on the sciatic nerve shear wave velocity ($P < 0.0001$; $_p\eta^2 = 0.658$). *Post hoc* tests revealed that the sciatic nerve shear wave velocity was 13.3 ± 7.9% lower ($p < 0.0001$) after the nerve stretching, while no significant changes (P = 0.486) were observed in the Control session.

*Sciatic nerve thickness*

Neither significant session × time interactions (P = 1), nor main effects of session (P = 0.747) or time (P = 0.538) were observed for the sciatic nerve stiffness. This result confirms that acute changes in shear wave velocity after stretch intervention were not influenced by the nerve thickness.

*Ankle torque*

A significant session × time (P = 0.042; $_p\eta^2 = 0.262$) interaction was observed at 100% of the pre-testing ankle ROM in dorsiflexion. However, post hoc tests showed no significant effect of the intervention on the passive torque (P = 0.074 and P = 0.416 for the Stretch and Control sessions, respectively), confirming that it was not significantly affected.

*Maximal ankle ROM in dorsiflexion*

A significant session × time interactions were observed on the maximal ankle ROM in dorsiflexion for both HIP-neutral and HIP-flexed positions [P = 0.010 ($_p\eta^2 = 0.384$) and $P < 0.0001$ ($_p\eta^2 = 0.644$), respectively]. For HIP-flexed, *post hoc* analysis revealed a significant increase (+6.4° ± 2.4°; $p < 0.0001$) in maximal ankle ROM in dorsiflexion immediately after the intervention performed during the Stretch session, but no significant changes were observed during the Control session (Figure 3A). Additionally, p*ost hoc* tests showed that the maximal ankle ROM in dorsiflexion assessed in HIP-neutral position increased after the intervention performed in the

Stretch session (+5.3° ± 4.2°; $p$ = 0.001), but no differences were observed in Control session (Figure 3B). A significant negative correlation (r = -0.571, P = 0.026) was observed between the percentage of sciatic nerve shear wave velocity decrease after the nerve stretch (Stretch session) and the percentage increase in maximal ROM in dorsiflexion assessed in HIP-flexed position (Figure 4).

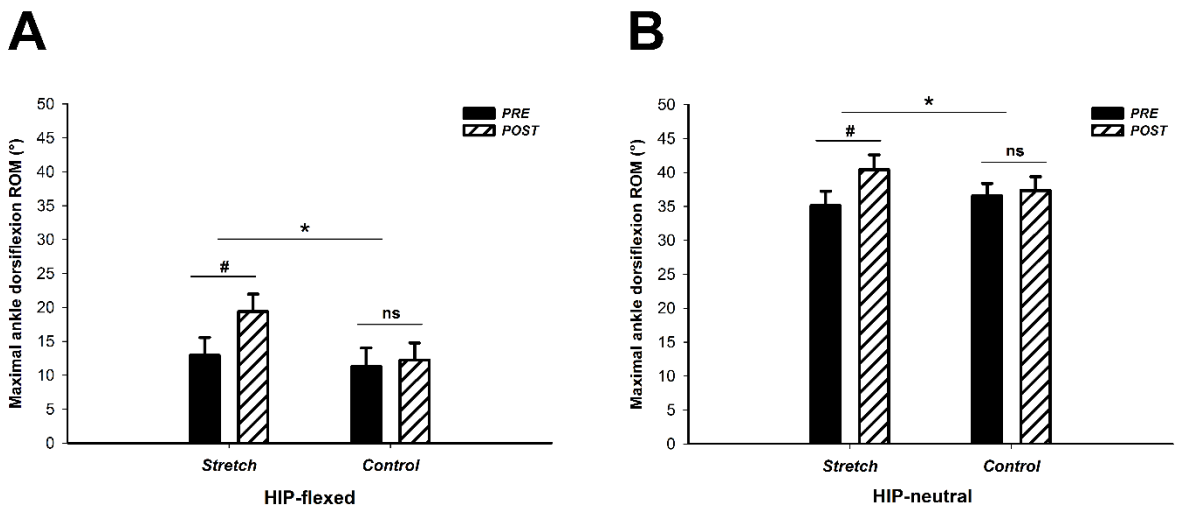


Figure 3. **A.** For the HIP-flexed position, the observed session × time interaction (*$p$ < 0.0001) demonstrates significant increase in the maximal dorsiflexion range of motion (ROM) after the intervention performed in the Stretch session ($^{\#}p$ < 0.0001); **B.** For the HIP-neutral, the interaction session × time (*$p$ = 0.010) shows that the maximum dorsiflexion ROM increases in the Stretch session ($^{\#}p$ = 0.001).

*RMS EMG*

Neither significant session × time interactions, nor main effects of session or time were observed for GL (1.17% ± 1.18%), SOL (2.85% ± 2.78%), ST (0.56% ± 0.58%) and TA (0.89% ± 1.35%) muscles.

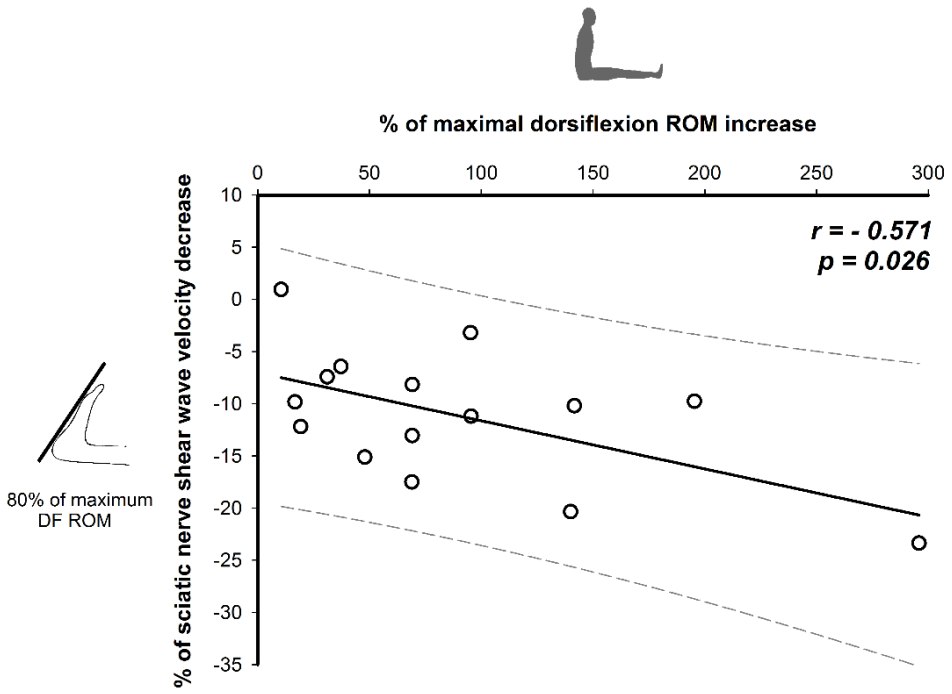


Figure 4. The effects of nerve stretching technique on local sciatic nerve stiffness and maximal ankle ROM in dorsiflexion. The negative correlation coefficient shows that the nerve stretching induced percentage increase in maximal ankle ROM in dorsiflexion measured in HIP-flexed position is correlated with the percentage decrease in sciatic nerve (Stretch session).

## DISCUSSION

The sciatic nerve stretching performed in this study was designed to directly manipulate the sciatic stiffness, and to examine the immediate effects of decreased nerve stiffness on the maximal ankle ROM in dorsiflexion. In accordance with our hypothesis, we observed that it is possible to alter the maximal ankle ROM in dorsiflexion without affecting the passive stiffness of plantar flexors muscles. This study provides the first *in-vivo* experimental evidence that stretching peripheral nerves is efficient to improve the maximal ROM at a joint, in particular when they are maximally lengthened (i.e. HIP-flexed position).

In the present study, the sciatic nerve stretch was effective to notably increase the maximal ankle ROM in dorsiflexion in the HIP-flexed (88.9 % ± 77.8 %), in which the sciatic nerve must be maximally stretched. Surprisingly, the maximal ROM has also improved, but with lower magnitude (17 % ± 15.1 %), in HIP-neutral position (Figure 3B), where the nerve load at the

maximal ROM in dorsiflexion is considerably lower (2). Although it is a long-held belief that the maximal ankle ROM in dorsiflexion assessed in HIP-neutral position is mainly explained by the tension developed by the plantar flexor muscles (36), our findings demonstrate that non-muscular structures, such as peripheral nerves, may also limit maximal ROM. Specifically, the sciatic nerve stretch can modify not only the nerve's mechanical behavior but also its sensory properties (i.e. nerve has dense sensory innervation being highly sensitive to stress/strain) increasing the stretch tolerance, even in a HIP-neutral position. This is shown in Figure 2 with the exponential increase in sciatic nerve stiffness observed during passive ankle dorsiflexion while the participants were positioned in HIP-neutral position. Using similar testing at the same HIP-neutral position, Andrade, et al. (2016) (3) showed that the steep increase in nerve stiffness occurs from ~50% of ankle's maximum dorsiflexion ROM (see Andrade, et al. (2016) (3)). Together, these findings demonstrate that the sciatic nerve is also loaded during ankle dorsiflexion in HIP-neutral position. Therefore, the decrease in sciatic stiffness observed in our study immediately after the stretch intervention may have played a role in the maximal ROM assessed in the HIP-neutral position.

We designed an experimental protocol to stretch the sciatic nerve without affecting plantar flexor muscles. It is accepted that lower limb position (i.e. hip, knee and ankle) may influence the loading of the sciatic nerve tract (1, 3, 5, 8, 17). Based on these cadaver and ultrasound findings, the sciatic nerve tract was stretched by manipulating the position of the pelvis, trunk and cervical spine. In regard to the position of the ankle joint during testing, although previous elastography studies have demonstrated that plantar flexor muscles are minimally stretched when the ankle is in neutral position (20, 24), it is unlikely that the stretching magnitude was sufficient to modify the muscles mechanical properties (14). This was confirmed by the fact that no significant changes of both ankle torque and muscle shear modulus were observed after the intervention in both the stretch and the control sessions; while the sciatic nerve stiffness decreased considerably immediately after the Stretch session. Taken together, these results support that the nerve stretching technique implemented in the present study targets the sciatic nerve with non-detectable changes in plantar flexors and knee flexors muscles. To our knowledge, this study is the first that experimentally validated stretching protocol dedicated to the nerve structures.

The specific physiological explanations for a change in nerve stiffness after the Stretch session remain somewhat speculative. Various *in-vitro* and *in-situ* works showed that nerves exhibit a non-

linear stress-strain behavior and time-dependent viscoelastic properties when stretched to a fixed strain (12, 23, 25, 32, 35). These findings suggest that nerve possesses a similar viscoelastic behavior to the muscle which can explain an acute decrease in stiffness while being stretched. In accordance with our hypothesis, the decreased sciatic nerve stiffness was accompanied with an overall increase in ROM in dorsiflexion, notably in HIP-flexed position. As no change occurred in plantar flexors muscles, we are confident that this change in maximal ROM in dorsiflexion is due to non-muscular structures. Although we can conclude that the sciatic nerve stiffness plays an important role on maximal ROM, the cumulative effect of other non-muscular structures (not-evaluated in this study) remains inconclusive. For example, like peripheral nerves, the *fasciae* of the lower limb and back is a continuous tissue, and thus it is also likely to affect the joint's motion when loaded by the hip flexion (9).

The main limitation of the present study is the position used to perform the shear-wave measurements (i.e. HIP-neutral). To determine the influence of sciatic nerve stiffness on maximal ankle ROM in dorsiflexion, it would have been more appropriate to measure the sciatic nerve stiffness in the HIP-flexed position that stretches the sciatic nerve more, and therefore contributes to a greater restriction of maximal ROM. However, after considerable piloting, we could not get reliable elastography measurements in this position. Therefore, we performed all the nerve stiffness evaluation in HIP-neutral as previously performed by Andrade, et al. (2016) (3). Additionally, our results were performed in one site of the sciatic nerve tract, which is further divided into two main branches. As it is known that nerves exhibit localized heterogeneity of tensile properties (29), the magnitude of the change in nerve stiffness might differ at different locations along the sciatic nerve.

Overall, the results of the present study show that a sciatic nerve stretching technique, by immediately decreasing the local sciatic nerve stiffness, can considerably increase the maximal ankle ROM in dorsiflexion without altering the plantar flexors stiffness. This suggests that nerve stretching techniques can play an important role in the prevention of nerve stretch injuries (e.g. in sports where high ROM is required), in the management of neuropathies or functional recovery after surgical repair. Interestingly, recent studies have shown that nerve stiffness is increased in pathologies where a joint's maximal ROM is thought to be decreased (11, 22). Further studies are required to determine whether frequent nerve stretching (i.e. across weeks) can improve joint

motion in sports and work activities, and subsequently help clinicians to design more optimal treatment protocols. Finally, we speculate that nerve stiffness may have an important role in preventing injuries, namely to myelinated nerves, and hence maintain optimal neural conduction (12, 30, 33). Further investigations are needed to determine the effects of the nerve stiffness and stretch training on its local conduction properties.

**Competing interests:** The authors declare no completing interests.

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## REFERENCES


1. Alshami AM, Babri AS, Souvlis T, Coppieters MW. Strain in the tibial and plantar nerves with foot and ankle movements and the influence of adjacent joint positions. *J Appl Biomech*. 2008;24(4):368-76.
2. Andrade RJ, Lacourpaille L, Freitas SR, McNair PJ, Nordez A. Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension. *Scandinavian journal of medicine & science in sports*. 2016;26(1):41-7.
3. Andrade RJ, Nordez A, Hug F et al. Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography. *Journal of biomechanics*. 2016;49(3):326-31.
4. Bercoff J, Tanter M, Fink M. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*. 2004;51(4):396-409.
5. Boyd BS, Topp KS, Coppieters MW. Impact of movement sequencing on sciatic and tibial nerve strain and excursion during the straight leg raise test in embalmed cadavers. *The Journal of orthopaedic and sports physical therapy*. 2013;43(6):398-403.
6. Brum J, Bernal M, Gennisson JL, Tanter M. In vivo evaluation of the elastic anisotropy of the human Achilles tendon using shear wave dispersion analysis. *Physics in medicine and biology*. 2014;59(3):505-23.
7. Cohen J. *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J. : L. Erlbaum Associates; 1988.
8. Coppieters MW, Alshami AM, Babri AS, Souvlis T, Kippers V, Hodges PW. Strain and excursion of the sciatic, tibial, and plantar nerves during a modified straight leg raising test. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2006;24(9):1883-9.
9. Cruz-Montecinos C, Gonzalez Blanche A, Lopez Sanchez D, Cerda M, Sanzana-Cuche R, Cuesta-Vargas A. In vivo relationship between pelvis motion and deep fascia displacement of the medial gastrocnemius: anatomical and functional implications. *Journal of anatomy*. 2015;227(5):665-72.
10. DeWall RJ, Slane LC, Lee KS, Thelen DG. Spatial variations in Achilles tendon shear wave speed. *Journal of biomechanics*. 2014;47(11):2685-92.
11. Dikici AS, Ustabasioglu FE, Delil S et al. Evaluation of the Tibial Nerve with Shear-Wave Elastography: A Potential Sonographic Method for the Diagnosis of Diabetic Peripheral Neuropathy. *Radiology*. 2016:160135.
12. Driscoll PJ, Glasby MA, Lawson GM. An in vivo study of peripheral nerves in continuity: biomechanical and physiological responses to elongation. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2002;20(2):370-5.
13. Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An KN. Validation of shear wave elastography in skeletal muscle. *Journal of biomechanics*. 2013;46(14):2381-7.

14. Freitas SR, Andrade RJ, Larcoupaille L, Mil-homens P, Nordez A. Muscle and joint responses during and after static stretching performed at different intensities. *European journal of applied physiology*. 2015;115(6):1263-72.

15. Gajdosik RL. Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clinical biomechanics*. 2001;16(2):87-101.

16. Gennisson JL, Deffieux T, Fink M, Tanter M. Ultrasound elastography: principles and techniques. *Diagnostic and interventional imaging*. 2013;94(5):487-95.

17. Greening J, Dilley A. Posture-induced changes in peripheral nerve stiffness measured by ultrasound shear-wave elastography. *Muscle & nerve*. 2016.

18. Helfenstein-Didier C, Andrade RJ, Brum J et al. In vivo quantification of the shear modulus of the human Achilles tendon during passive loading using shear wave dispersion analysis. *Physics in medicine and biology*. 2016;61(6):2485-96.

19. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*. 2000;10(5):361-74.

20. Hirata K, Kanehisa H, Miyamoto-Mikami E, Miyamoto N. Evidence for intermuscle difference in slack angle in human *triceps surae*. *Journal of biomechanics*. 2015;48(6):1210-3.

21. Hug F, Tucker K, Gennisson JL, Tanter M, Nordez A. Elastography for Muscle Biomechanics: Toward the Estimation of Individual Muscle Force. *Exerc Sport Sci Rev*. 2015;43(3):125-33.

22. Kantarci F, Ustabasioglu FE, Delil S et al. Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. *Eur Radiol*. 2014;24(2):434-40.

23. Kwan MK, Wall EJ, Massie J, Garfin SR. Strain, stress and stretch of peripheral nerve. Rabbit experiments in vitro and in vivo. *Acta Orthop Scand*. 1992;63(3):267-72.

24. Le Sant G, Nordez A, Andrade R, Hug F, Freitas S, Gross R. Stiffness mapping of lower leg muscles during passive dorsiflexion. *Journal of anatomy*. 2017;230(5):639-50.

25. Ma Z, Hu S, Tan JS, Myer C, Njus NM, Xia Z. In vitro and in vivo mechanical properties of human ulnar and median nerves. *J Biomed Mater Res A*. 2013;101(9):2718-25.

26. Mitchell B, Bressel E, McNair PJ, Bressel ME. Effect of pelvic, hip, and knee position on ankle joint range of motion. *Phys Ther Sport*. 2008;9(4):202-8.

27. Nordez A, Gross R, Andrade R et al. Non-Muscular Structures Can Limit the Maximal Joint Range of Motion during Stretching. *Sports Med*. 2017.

28. Nordez A, McNair P, Casari P, Cornu C. Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. *International journal of sports medicine*. 2008;29(5):414-8.

29. Phillips JB, Smit X, De Zoysa N, Afoke A, Brown RA. Peripheral nerves in the rat exhibit localized heterogeneity of tensile properties during limb movement. *The Journal of physiology*. 2004;557(Pt 3):879-87.

30. Rickett T, Connell S, Bastijanic J, Hegde S, Shi R. Functional and mechanical evaluation of nerve stretch injury. *J Med Syst*. 2011;35(5):787-93.

31. Salsich GB, Mueller MJ, Sahrmann SA. Passive ankle stiffness in subjects with diabetes and peripheral neuropathy versus an age-matched comparison group. *Physical therapy*. 2000;80(4):352-62.

32. Shah SB. Tissue Biomechanics: Whales Have Some Nerve. *Curr Biol*. 2017;27(5):R177-R9.

33. Shim HY, Lim OK, Bae KH, Park SM, Lee JK, Park KD. Sciatic nerve injury caused by a stretching exercise in a trained dancer. *Ann Rehabil Med*. 2013;37(6):886-90.

34. Urban LM, MacNeil BJ. Diagnostic Accuracy of the Slump Test for Identifying Neuropathic Pain in the Lower Limb. *The Journal of orthopaedic and sports physical therapy*. 2015;45(8):596-603.

35. Wall EJ, Kwan MK, Rydevik BL, Woo SL, Garfin SR. Stress relaxation of a peripheral nerve. *J Hand Surg Am*. 1991;16(5):859-63.

36. Weppler CH, Magnusson SP. Increasing muscle extensibility: a matter of increasing length or modifying sensation? *Physical therapy*. 2010;90(3):438-49

# STUDY 4

## Localized muscle and nerve stiffness adaptations following a 12-weeks of specific stretch training

**Andrade RJ**, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Gross R, Quillard JB & Nordez A. (In preparation)

LOCALIZED MUSCLE AND NERVE STIFFNESS ADAPTATIONS FOLLOWING A 12-WEEKS OF SPECIFIC STRETCH TRAINING

# ABSTRACT

Although it exists strong evidence that chronic stretching protocols improve joint maximal range of motion (ROM), the underlying mechanisms are still debated. It has been suggested that (1) adaptations to protocols shorter than 8 weeks only occur at sensory level, and (2) non-muscular tissues such as peripheral nerves play an important role on joint ROM. This study aimed to examine the effect of a 12-week stretching protocol designed to target either muscular or nerve structures on *triceps surae* and sciatic nerve stiffness.

Participants were assigned to one of three groups: sciatic stretching (Sciatic STR, n=20), *triceps surae* stretching (muscle STR, n=21), or control (CON, n=17). Passive maximal ankle dorsiflexion ROM was assessed with the hip flexed (HIP-flexed) and neutral (HIP-neutral, 0°). Both muscle and nerve stiffness were estimated using shear wave elastography in HIP-neutral.

Sciatic STR induced an increase in ankle ROM in both HIP-neutral and HIP-flexed conditions (+3.8±4.9°; +8.0±5.2°, respectively; both $P<0.001$), and a decrease in sciatic nerve stiffness (-19.1±15.3%; $P < 0.0001$) in the absence of any change in *triceps surae* stiffness (all $P=1$). Muscle STR induced an increase in ankle ROM in HIP-neutral position (+9.4° ± 3.8°; $P < 0.0001$) and a decrease in muscle stiffness (heterogeneous response; all $P<0.03$), without altering sciatic nerve stiffness (all $P=1$). Ankle passive torque at a given angle decreased after muscle STR but remained unchanged after sciatic STR.

### *Contribution to this doctoral research:*

- Both muscle and nerve stretching protocols are effective at increasing the maximal joint ROM, with specific mechanical adaptations in the targeted tissues.
- While muscle stretching only alters the maximal joint ROM for the body posture used during the intervention (HIP-neutral), the nerve stretching increases the maximal joint ROM in different body postures (i.e., HIP-flexed and HIP-neutral).

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**Keywords:** supersonic shear imaging; shear wave velocity; elastography; ultrasound; sciatic; nerve stretch; neurodynamics; range of motion; ankle

# Localized muscle and nerve stiffness adaptations following a 12-weeks of specific stretch training

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**Article Type:** Original Study

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**Keywords:** supersonic shear imaging; shear wave velocity; elastography; ultrasound; sciatic; nerve stretch; neurodynamics; range of motion; ankle

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# INTRODUCTION

Stretching human skeletal muscles is commonly used in sports and clinical practice with the aim of increasing muscle-tendon extensibility and joint range of motion (ROM). Although it is widely accepted that chronic stretching interventions induce an increase in maximal ROM, it exists evidence that stretching interventions shorter than 8 weeks do not alter the joint passive torque-angle relationship and muscle-tendon unit mechanical properties (Weppler & Magnusson, 2010; Freitas *et al.*, 2017). This lack of mechanical adaptations led to the conclusion that increased ROM is mostly explained by an increase in stretch tolerance (Weppler & Magnusson, 2010; Freitas *et al.*, 2017).

However, three main limitations can be outlined in respect to the studies who reported absence of joint and muscle-tendon unit mechanical changes following chronic stretching. First, studies were implemented to relatively short stretch training periods (<8 weeks) (Weppler & Magnusson, 2010; Freitas *et al.*, 2017). Therefore, it remains possible that the duration of the stretching intervention was not sufficient to trigger mechanical adaptations within the muscle-tendon unit (Freitas *et al.*, 2017; Nordez *et al.*, 2017). Second, the mechanical properties of muscle-tendon units were inferred from passive torque that represents the global resistance at the joint level (Magnusson, 1998; Gajdosik, 2001). Because stretching interventions may selectively load muscles within a muscle group and within the same muscle (Hirata *et al.*, 2016; Le Sant *et al.*, 2017), passive torque may not be sensitive enough to detect tensile changes within individual structures. For instance, Akagi and Takahashi (2013) showed a decrease of local *gastrocnemii* transverse stiffness after an acute stretching of plantar flexors stretching in the absence of any change in ankle passive torque at a given angle. Third, non-muscular structures such as peripheral nerves were proposed as possible candidates to influence the maximal joint ROM (Andrade *et al.*, 2016a; Neto *et al.*, 2017; Nordez *et al.*, 2017). Recently, our research group (Andrade et al. submitted; study 3 of this thesis) observed that the maximal ankle dorsiflexion ROM can be acutely increased by decreasing the sciatic stiffness, in the absence of any change in tensile properties of plantar flexors muscles and the ankle passive torque.

Despite chronic stretching has become a widely accepted means of treating clinical conditions or decrease the tissue injury risk (Ekstrand *et al.*, 1983; Harvey & Herbert, 2002; Witvrouw *et al.*, 2003; Pin *et al.*, 2006), it remains unclear whether it is possible to induce lasting stiffness changes in either muscle or non-muscular structures, and if such individual adaptations are reflected on global passive joint torque adaptations (Freitas *et al.*, 2017; Nordez *et al.*, 2017; Shah, 2017). To date, no previous study has examined *in-vivo* the mechanical responses of peripheral nerves to chronic and if they would affect functional outcomes such as maximal joint ROM.

This study aimed to examine the passive mechanical adaptations of muscle and nerve tissues after long-term stretching protocols designed to target preferentially each structure. To this end, the joint resistance to stretch (i.e. passive torque) and the local passive stiffness of both sciatic nerve and plantar flexors muscles measured using ultrasound shear wave elastography were assessed before and after 12-weeks of two stretch training either targeting the sciatic nerve (Sciatic STR) or the plantar flexors (Muscle STR). We hypothesized that the chronic stretching protocols would increase the maximal joint ROM, with mechanical changes in either the nerve or muscular tissues depending on the stretching protocol used to target each tissue.

## MATERIALS AND METHODS

### Participants

Sixty healthy and asymptomatic volunteers (age: 20.5 ± 2.0 years, height: 172.2 ± 9.1 cm, weight: 63.8 ± 9.0 kg) participated in this study. Participants were recruited in local Faculty of Sports Sciences, between September and October 2017 by the use of posted flyers and personal contact. Exclusion criteria comprised (i) recent (< 3 years) musculoskeletal injury/disability in spine or lower limbs (ii) severe cardiovascular, neurological or psychiatric disease; (iii) positive straight leg raising test to exclude sciatic nerve or nerve roots pathology; and (iv) permanent drug intake. Inclusion criteria comprised (i) healthy males or females, (ii) aged between 18 and 30 years, (iii) not engaged in any flexibility training. After successfully completed the recruitment session, participants were randomly assigned to either sciatic stretching (Sciatic STR; n = 21; 12 males and 9 females), muscle stretching (muscle STR; n = 21; 10 males and 11 females), or control (CON, n

= 18; 9 males and 9 females) groups, as described on the Figure 1. Participants' characteristics are presented in Table 1. Variance analytical group comparisons revealed no significant differences between the groups for the demographic, anthropometric and baseline ROM data. All participants reported to practice more than 8h/week of intense physical activity before and during the period of this study. Participants were informed about methods used in this study before providing informed written consent, and were asked to avoid intense exercise 48 h prior to the testing sessions performed before and after the stretch training. The Institutional Ethics Committee approved the study, and all procedures conformed to the Declaration of Helsinki with its recent modification of Fortaleza (2013).

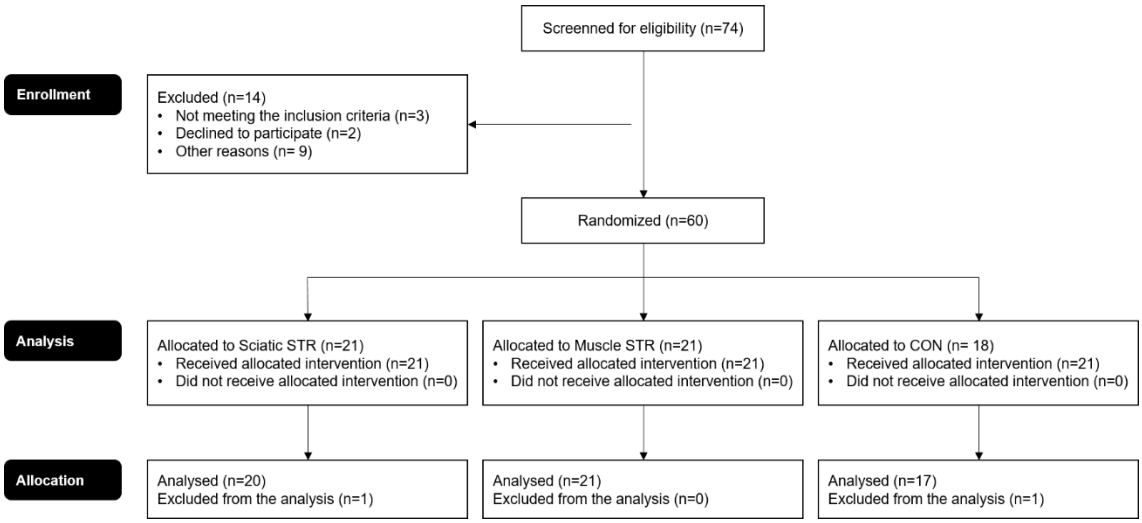



Figure1. Flow chart of the trial.

Table 1: Descriptive data for the three groups. Demographic, anthropometric and baseline data of the sample including variance analytical group comparisons and according P-values.

| | **Total sample** | **Nerve STR (n = 21)** | **Muscle STR (n = 21)** | **CON (n = 18)** | **P-value** |
|---|---|---|---|---|---|
| **Age (years)** | 20.5 ± 2.0 (19.9 – 21.0) | 19.5 ± 1.1 (18.9 – 20.1) | 21.0 ± 2.4 (19.7 – 22.1) | 21.1 ± 2.0 (20.0 – 22.1) | 0.096 |
| **Gender (males/females)** | 31/29 | 12/9 | 10/11 | 9/9 | 0.097 |
| **Height (cm)** | 172.2 ± 9.2 (170.2 – 174.7) | 174.7 ± 8.5 (170.2 – 179.2) | 170.6 ± 9.3 (166 – 175.3) | 171.5 ± 8.5 (167.3 – 175.7) | 0.366 |
| **Body mass (kg)** | 63.7 ± 9.0 (61.3 – 66.0) | 65.6 ± 8.8 (61.2 – 70.0) | 62.8 ± 9.6 (58.0 – 67.6) | 62.2 ± 8.9 (57.7 – 66.6) | 0.480 |
| **Baseline ROM HIP-neutral (°)** | 38.3 ± 7.5 (34.6 – 41.1) | 37.8 ± 6.9 (34.6 – 41.1) | 36.9 ± 7.6 (34.0 – 41.7) | 40.6 ± 8.1 (36.5 – 44.8) | 0.564 |
| **Baseline ROM HIP-flexed (°)** | 11.4 ± 10.8 (4.5 – 13.1) | 8.8 ± 9.1 (4.5 – 13.1) | 13.9 ± 10.7 (10.5 – 21.6) | 11.4 ± 12.5 (5.0 – 17.7) | 0.139 |

Data are means ± standard deviation (95% confidence interval); ROM, range of motion.

**Stretch training**

Sciatic STR and muscle STR participants were enrolled in a 12-weeks stretch training protocol, performed five times per week between 10:00 am and 3:00 pm. In each session participants performed two stretching exercises according with their allocated group (sciatic STR or muscle STR). Each stretching exercise was maintained 45 s and performed five times. Participants were instructed to start the following repetition immediately after release the previous one (< 5-s interval). Duration of stretch exposure was identical between muscle and sciatic STR groups. Each session lasted 450 s, leading to a total stretch duration of 7.5 h over the 12-weeks training period.

Stretching exercises were supervised by experienced examiners in order to ensure that the maximal stretch tolerance, defined as the onset of pain, was reached. Specifically, the stretch exercises were chosen as they are commonly used in clinical practice and sports.

Each session of the sciatic STR group was composed by the following exercises performed in a randomized order: (1) from a seated position, the examiner passively flexed the hip towards a long sitting position, followed by lumbar, thoracic and cervical flexion to the end of range of motion. The knees remained in full extension, and the ankle angles were maintained in neutral position (0°) to minimize the stretch of the plantar flexor muscles (Figure 2-A). (2) The participant was initially laid supine with the knees in full extension, and then participants' legs were passively raised by an examiner (i.e. hip flexion) with the ankles in neutral position. For both exercise, the ankles were placed in neutral position (Figure 2-B). The effectiveness of these exercises to target acute changes in sciatic nerve mechanical properties have been demonstrated (Andrade *et al.*, submitted; study 3 of this doctoral research).

Participants from the muscle STR group performed the two following plantar flexors stretch exercises in a randomized order: (1) the participant was instructed to stand on the step, with the knees in full extension with their heels hanging over the edge, and the forefeet supporting the weight. Then, the stretch was performed by dropping the heels slowly (both sides together) towards the ground until the maximal stretch tolerance (Figure 2-C). (2) The same exercise was performed with the knee flexed between 50° and 90° (Figure 2-D). These knee angles were chosen to specifically target both gastrocnemii (knees fully-extended) and soleus muscles (knees flexed) (Le Sant *et al.*, 2017).

Participants of CON groups were instructed to not enroll in any specific flexibility training, and to maintain the same level of physical activity for the duration of the stretch training protocol.


Figure 2. Stretching exercises performed during the stretch training. **A.** and **B.** correspond to stretching exercises performed to stretch the sciatic nerve by the "sciatic STR group". **C.** and **D.** are the stretching exercises performed by the "muscle STR group" to target the plantar flexors stretching.

## Ergometer

An isokinetic dynamometer (System 3; Biodex Medical Systems, NY, USA) was used to perform passive ankle rotations and to measure the ankle torque during testing sessions performed pre- and post-stretch training period. The lateral malleolus, considered as an estimate of the ankle center of rotation, was aligned with the axis of the ergometer. The neutral position of the ankle (0º) was defined as an angle of 90º between the footplate and the shank. The foot was firmly strapped to minimize the potential heel displacement from the dynamometer platform during the ankle rotations. All measurements were performed on the right ankle joint.

Angle and torque data were collected at 100 Hz with an analogue/digital converter (Biopac Systems Inc., Goleta, USA). Two hip positions were tested: flexed at 90° (HIP-

flexed; Figure 3-A) or neutral (HIP-neutral, 180°; Figure 3-B). A standard goniometer (MSD, Londerzeel, Belgium) was used to setup the hip angle. For both conditions, knee was placed in full extension (180°).

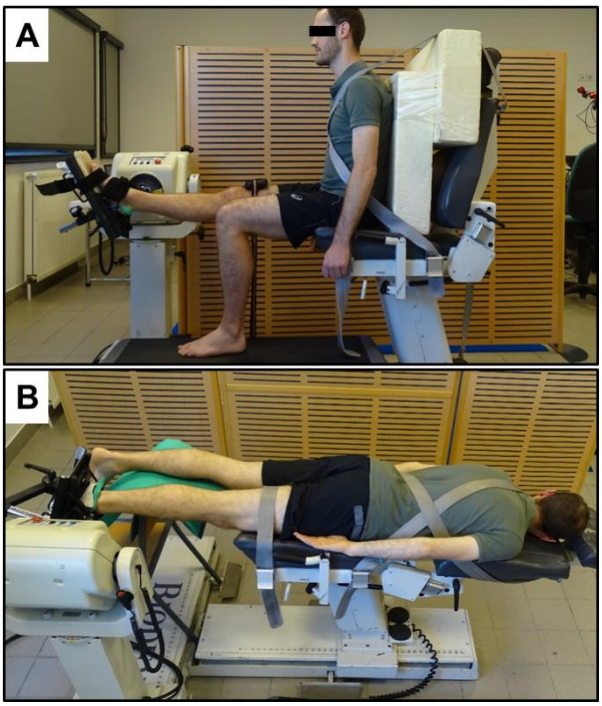



Figure 3. Testing positions used to assess the maximal dorsiflexion range of motion; **A.** HIP-flexed position; **B.** HIP-neutral position.

**Ultrasound recordings**

Because our previous pilot testing demonstrated that nerve elastography measurements were not reliable in the HIP-flexed positions (unpublished data; please see pilot study of this doctoral research), all the elastography assessments were performed in the HIP-neutral position. The shear wave velocity of the sciatic nerve, *gastrocnemius medialis* (GM), *gastrocnemius lateralis* (GL) and *soleus* (SOL) was assessed using an Aixplorer ultrasound scanner (version 6.1; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer (4-15 MHz for GM, GL muscles, and 2-10 MHz for the sciatic nerve and SOL muscle; Super Linear, Aix-en- Provence, France).

The muscle and nerve structures regions of interest (ROI) were first identified in B-mode images, with participants laying prone, knee in full extension, and the ankle in a neutral position (0°). Three location landmarks were first identified using B-mode to assess the neural structures: (i) the halfway point between the ischial tuberosity and the greater trochanter, (ii) the division of the sciatic nerve in the popliteal fossa, and (iii) the

lateral femoral condyle. Then, sciatic nerve was divided in two equidistant proximal ($sciatic_{proximal}$) and distal ($sciatic_{distal}$) regions between points (i) and (ii). Tibial (TBL) nerve was assessed between the locations points (ii) and (iii). SCI and TBL were considered as sciatic nerve tract. The transducer was positioned parallel to the nerve fibers (Andrade *et al.*, 2016b).

The shear wave velocity measurements of GM and GL muscles were performed at three equidistant regions (i.e. 33% of muscle length: proximal, intermediate and distal segments), and in two equidistant regions for the SOL (i.e. proximal and distal), as described by (Le Sant *et al.*, 2017). Two location landmarks were identified to perform these measurements: (i) mid-distance between lateral and medial malleoli, and (ii) popliteal fossa. $SOL_{distal}$ and $SOL_{proximal}$ were assessed at 10% and 50% from the distance starting from the point (i) to point (ii), respectively. The transducers were hand-held secured to the thigh (SCI and TBL) or the lower leg (GM, GL and SOL) by an experienced ultrasonographer. Minimal tissue compression was performed throughout assessments.

The following shear wave elastography acquisition parameters were set: penetration mode, 100% opacity, no temporal smoothing (persistence=OFF), and intermediate spatial smoothing of 5/9. The maps of the shear modulus were obtained at 1 sample/s and with a spatial resolution of 1 × 1 mm. An analog trigger signal originating from the ultrasound scanner at each elastography measurement was used to synchronize shear wave velocity data with mechanical and surface electromyography (EMG) data.

**Electromyography (EMG)**

Myoelectrical activity was assessed using surface EMG to ensure that the lower leg muscles remained inactive during the measurements. A pair of surface electrodes (Kendall 100 Series Foam Electrodes, Covidien, Massachusetts, USA) was placed over the GM and SOL muscles at the location recommended by the Surface EMG for Non-Invasive Assessment of Muscles guidelines (Hermens *et al.*, 2000). Adjustments were performed on the location of the electrodes when ultrasound measurements were performed on the intermediate position of the GM. EMG data were acquired simultaneously with the mechanical data (MP35, Biopac Systems Inc., Goleta, USA) at a sampling rate of 1 kHz. The EMG signals were amplified (Gain = 1000) and band-pass

filtered between 5 and 500 Hz.

**Pre- and post-training measurements protocol**

Thirty minutes prior to the each testing session, participants were familiarized with the testing set-up and maximal ankle dorsiflexion ROM assessments (static stretching was avoided). Pre- and post-training testing sessions were performed 24-h prior the first stretch session, and 24-h following the last stretch session, respectively. Both testing sessions were conducted at the same period of the day (± 3h). At the start of each experimental session, five conditioning ankle rotations were performed (at 5°/s) in the HIP-neutral position, between 40° of plantar flexion and 15° of dorsiflexion (Nordez *et al.*, 2008). Thereafter, the testing sessions were divided in 4 steps.

First, the passive maximal ankle dorsiflexion ROM was assessed in both HIP-neutral and HIP-flexed positions (randomized order). Participants were asked to completely relax with their eyes closed, while the ankle was passively rotated at 2°/s from 40° plantar flexion toward maximal dorsiflexion. When the participants reached their maximum tolerable stretch limit (Andrade *et al.*, 2016a), they pressed a button that immediately released the footplate, and hence the associated stretch on the soft tissue structures. For each hip position, participants performed two trials with 1-min rest in-between. As recommended by Blazevich *et al.* (2014), the greatest ankle ROM in dorsiflexion attained during these trials was retained for further analysis. For the HIP-neutral position, participants laid prone on the dynamometer with their knee fully extended and the contralateral limb in a relaxed position. For the HIP-flexed position, participants were seated with the right hip flexed at 90º, the knee in full extension, and the contralateral hip and knee flexed at 90°. The chest and the waist were strapped to minimize trunk motion during ankle rotations. A very good repeatability was observed between ROM trials, with standard error of measurement (SEM) of 1.02° and 1.11° for HIP-neutral and HIP-flexed, respectively.

Second, elastography measurements were randomly performed on GM (×3 regions), GL (×3 regions), SOL (×2 regions), and sciatic nerve tract (×3 regions) while the ankle was passively rotated (2°/s) from 40° of plantar flexion to the previously determined maximal dorsiflexion ROM in the HIP-neutral position. The order of the measurement was randomized over the 11 passive stretches.

Third, two ultrasound B-mode images were performed for each muscle of *triceps surae* ($GM_{intermediate}$, $GL_{intermediate}$, and $SOL_{proximal}$) in order to assess their architecture: fiber length, pentation angle and thickness. Additionally, two ultrasound B-mode images were acquired in sciatic nerve tract ($sciatic_{proximal}$, $sciatic_{distal}$ and tibial) to assess cross nerve section area and thickness. All of these measurements were performed with the ankle at neutral position (0°). **Please note that the adaptations in nerve and muscle architecture are currently being processed.** As we believe that these results are important to explain the source of the stiffness adaptations, they will be presented in the oral defense.

Finally, two maximal isometric plantarflexions were performed with the ankle in a neutral position (0°) with the participants in supine, in order to normalize the EMG signals. There was a 1-min rest interval between each maximal effort trials.

**Data analysis**

Two participants (sciatic STR: 1; CON: 1) were excluded for data analysis because of imaging acquisition artifacts (1) and serious knee ligament injury that occurred during the protocol (1). Thus, data from 58 participants (sciatic STR: 20; muscle STR: 21; CON: 17) were analyzed.

MATLAB scripts (The MathWorks Inc., Natick, USA) were used to all data processing. Angle and torque signals were low-pass filtered (10 Hz) with a second-order Butterworth filter. The torque was further corrected for gravity.

Both muscle and nerve stiffness estimation were based on shear wave velocity (Vs) quantification. The Vs is directly linked to the shear modulus ($\mu$), $\mu = \rho Vs^2$, where $\rho$ is the estimated density of soft tissues (1000 kg/m$^3$) (Bercoff *et al.*, 2004). Since shear wave dispersion analysis has shown that shear wave propagation is guided in Achilles tendon, the tendon thickness is likely to influence the group shear wave velocity that cannot be directly related to the shear modulus (Brum *et al.*, 2014; Helfenstein-Didier *et al.*, 2016). Although sciatic nerve is less stiff than Achilles tendon and thus the guided shear wave propagation is likely less problematic (DeWall *et al.*, 2014; Andrade *et al.*, 2016b), no study has applied this shear wave dispersion analysis to peripheral nerves. Therefore, our results are reported as shear wave velocity (Andrade *et al.*, 2016b). Clip videos were

exported from the Aixplorer's scanner on 'mp4' format, and then sequenced in 'jpeg' images. The image processing converted each pixel of the color map into a shear wave velocity value based on the recorded color scale (scale = 0-18.3 m/s). A region of interest (ROI) was first defined on the first map as the largest muscle or nerve area. Then this ROI was manually tracked image-by-image. Each ROI was then inspected for artifacts (i.e. saturation areas). If artifacts were present in any of the images to be analyzed within a recording, the ROI was reduced in size to exclude the area of artifact from all images within that recording (Andrade *et al.*, 2016b). The shear wave velocity data extracted from this ROI was averaged to obtain a representative value.

The sciatic nerve (×3 regions), GM (×3 regions), GL (×3 regions) and SOL (×3 regions) shear wave velocity, ankle torque and RMS EMG (×2 muscles) were calculated every 2°, from 40° of plantar flexion to the maximal ankle ROM in dorsiflexion.

In order to standardize range of motion to a percentage of maximum, a linear interpolation method was used to calculate the shear wave velocity of targeted nerves and muscles, and ankle torque every 5% of ankle ROM.

The root mean square of the EMG signal (RMS EMG) was calculated every second over 500-ms moving windows throughout the testing procedures. Artifacts originating from the supersonic push beam (1 sample/s) were discarded from EMG. The RMS EMG values were normalized to that recorded during maximal voluntary isometric contractions.

The maximal ankle ROM in dorsiflexion attained individually in the pre-testing was used to examine the pre- to post-effects of stretch training. All the assessed parameters (nerve and muscle shear wave velocity, ankle torque and RMS EMG values) were determined using a linear interpolation method at this ankle angle, and then used for statistical analysis purposes.

**As it was said previously, architecture data are currently being processed.** The length of muscle fibers, fiber pennation angle and muscle thickness were calculated simultaneously from B-mode images using custom-written MATLAB scripts. *Gastrocnemius medialis*, *gastrocnemius lateralis* and soleus were analyzed. Specifically, two different images were processed for each muscle. Briefly, two straight lines were digitally superimposed on each image to track superficial and deep aponeuroses. The fiber length was measured as the length of the fiber (straight line distance) between their

intersections with the aponeuroses. When exceeded the ultrasound field of view, an extrapolation of the fascicle length was performed using trigonometry (Randhawa *et al.*, 2013). In each image, three distinct fascicles were tracked (proximal, intermediate and distal), and the average length between the three fibers was used for statistical analysis. Pennation angle was calculated as the angle formed between the deep aponeurosis and a fascicle. Muscle thickness was calculated as the average of the straight distance between superficial and deep aponeuroses. In addition, nerve thickness was calculated using B-mode ultrasound images of $sciatic_{proximal}$, $sciatic_{distal}$ and tibial. It was calculated as a mean of six different straight distances between nerve boundaries (i.e. superficial and deep epineurium). Finally, nerve cross-sectional area was tracked three times, calculated as the area of nerve in a transversal B-mode image, and the average was used for further analysis.

**Statistics**

The IBM SPSS software (version 20.0; IBM Corporation, New York, USA) was used for the statistics procedures. Distributions consistently passed the Shapiro-Wilk normality test. All data are thus reported as mean ± standard deviation (SD).

One three-way ANOVA [independent factor was group (sciatic STR, muscle STR, CON); within-subject factors were testing position (HIP-neutral, HIP-flexed) × time (pre, post)] was performed to identify changes in maximal ROM in dorsiflexion after the stretching training. Testing position and time were treated as within factors.

One three-way ANOVA [independent factor was group (sciatic STR, muscle STR, CON); within-subject factors were muscle ($GM_{proximal}$, $GM_{intermediate}$, $GM_{distal}$, $GL_{proximal}$, $GL_{intermediate}$, $GL_{distal}$, $SOL_{proximal}$, $SOL_{distal}$) × time (pre, post)] was performed to examine the effect of the intervention on shear wave velocity of the tested muscle. Additionally, one three-way ANOVA [independent factor was group (sciatic STR, muscle STR, CON); within-subject factors were nerve ($sciatic_{proximal}$, $sciatic_{distal}$, tibial) × time (pre, post)] was performed to test the effect of the intervention on the sciatic nerve shear wave velocity. Two two-way ANOVAs [independent factor was group (sciatic STR, muscle STR, CON); within-subject was time (pre, post)] were performed to examine the effect of the stretch training on ankle passive torque in both HIP-flexed and HIP-neutral testing positions.

This analysis was performed on the passive torque values measured at the maximal ankle ROM in dorsiflexion during pre-testing.

The effects of stretch training on the RMS EMG during the whole cycle and RMS EMG at maximal dorsiflexion ROM were tested by using two three-way ANOVA [independent factor was group (sciatic STR, muscle STR, CON); within-subject factors were muscle (GM, SOL) × time (pre, post)].

The partial eta square ($_p\eta^2$) values were reported as a measure of the effect size of the ANOVA's findings. Small, medium and large effects were considered for $_p\eta^2 = 0.01$, $_p\eta^2 = 0.06$ and $_p\eta^2 = 0.14$, respectively (Cohen, 1988). Post hoc analyses subsequent to the ANOVAs were performed when appropriated, using the Bonferroni correction for multiple comparisons. The statistical significance was set at $P < 0.05$.

## RESULTS

*Maximal ankle dorsiflexion ROM*

A significant group × hip position × time interaction was observed for the maximal ankle ROM in dorsiflexion ($P < 0.0001$, $_p\eta^2 = 0.6$; Figure 4). *Post hoc* analysis performed in sciatic STR group revealed a significant increase in maximal ankle ROM in dorsiflexion following the stretch intervention for both HIP-neutral (+3.8±4.9°; $P = 0.001$) and HIP-flexed positions (+8±5.2°; $P < 0.0001$). For the muscle STR group, the maximal ankle ROM in dorsiflexion assessed in HIP-neutral position increased notably after the intervention (+9.4° ± 3.8°; $P < 0.0001$), but no differences were observed in HIP-flexed position (-2.1° ± 3.8°; $P = 0.97$). Additionally, *post hoc* tests performed for CON group showed no significant effect of time on maximal ROM in dorsiflexion neither for HIP-neutral (+0.8° ± 3.7°; $P = 1$) nor HIP-flexed positions (-1.6° ± 3.3°; $P = 1$) .

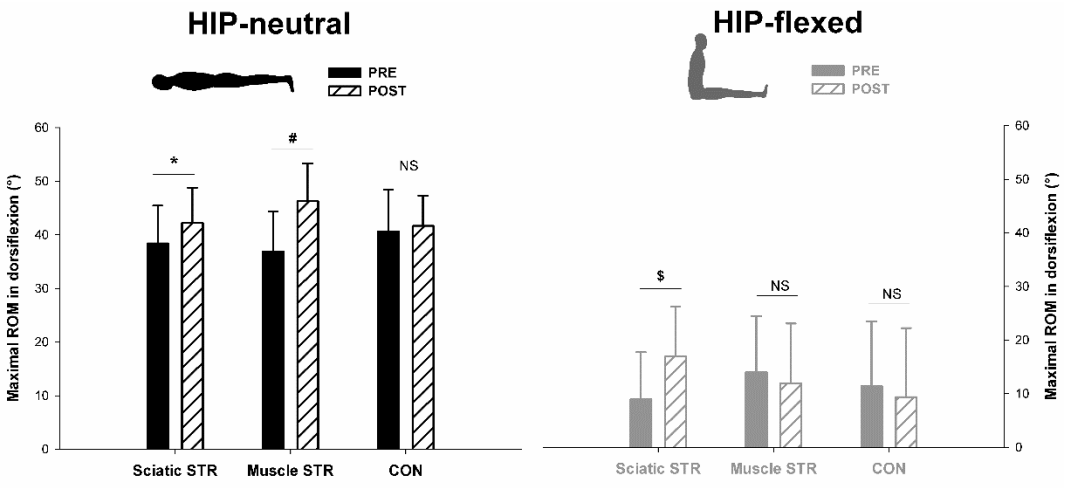



Figure 4. Effects of stretching regimens on maximal ROM assessed in both HIP-neutral and HIP-flexed positions. A group × hip position × time interaction was observed for the maximal ankle ROM in dorsiflexion ($P < 0.0001$). For the HIP-neutral position, *post hoc* analysis demonstrates significant increase in maximal ankle ROM in dorsiflexion following the sciatic STR ($^{*}P = 0.001$) and muscle STR ($^{\#}P < 0.0001$) interventions, while no changes are observed CON ($^{NS}P = 1$). For the HIP-flexed position, *post hoc* analysis shows significant increase in maximal ankle ROM in dorsiflexion following the sciatic STR intervention ($^{\$}P < 0.0001$), in the absence of changes in both muscle STR ($^{NS}P = 0.97$) and CON ($^{NS}P = 1$).

*Muscle shear wave velocity*

A significant muscle × group × time interaction effect was observed for the shear wave-velocity measured at maximum dorsiflexion ROM of pre-testing ($P = 0.03$, $_{p}\eta^2 = 0.1$). *Post hoc* tests revealed that shear wave velocity decreased significantly for $GM_{intermediate}$ (-14.3±12.9%; $P < 0.0001$), $GM_{distal}$ (-15.2±13.3%; $P < 0.0001$) and $GL_{proximal}$ (-13.8± 22.2%; $P < 0.03$) following the stretch intervention performed by muscle STR group, while no significant differences were found for the other locations (P values > 0.22). There were no significant pre- to post-training changes on muscle shear wave velocity for both Sciatic STR (all $P = 1$) and CON (all $P = 1$) groups (Table 2).

Table2. Pre to post-training adaptations on muscular and neural tissues in accordance with the experimental group: sciatic STR, muscle STR and CON.

| | **Sciatic STR[a,b,c] (ankle angle: 34.9° ± 6.2°)** | | | | **Muscle STR[a,b,c] (ankle angle: 35° ± 6.8°)** | | | | **CON[a,b,c] (ankle angle: 39.2° ± 6.4°)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Region** | PRE[a,b,c] | POST[a,b,c] | Change, % | P | PRE[a,b,c] | POST[a,b,c] | Change, % | P | PRE[a,b,c] | POST[a,b,c] | Change, % | P |
| **Muscle shear wave velocity** | | | | | | | | | | | | |
| **$GM_{proximal}$ (m/s)[a]** | 8.7 ± 1.8 | 8.8 ± 2.2 | 1.6 ± 16.4 | 1 | 9.3 ± 1.9 | 8.4 ± 2.3 | -9.3 ± 17.9 | 1 | 9.8 ± 1.6 | 10.5 ± 1.9 | 7.4 ± 14.4 | 1 |
| **$GM_{intermediate}$ (m/s)[a]** | 9.8 ± 2.1 | 9.8 ± 2.4 | 0.4 ± 13.6 | 1 | 10.7 ± 1.9 | 9.2 ± 2.1 | -14.3 ± 12.9 | < 0.0001 | 11.2 ± 1.6 | 11.6 ± 1.7 | 4.7 ± 8.5 | 1 |
| **$GM_{distal}$ (m/s)[a]** | 10.3 ± 2 | 9.8 ± 2.4 | -4 ± 17.9 | 1 | 11.2 ± 2.2 | 9.7 ± 2.8 | -15.2 ± 13.26 | < 0.0001 | 12.2 ± 1.9 | 12.8 ± 2.6 | 4.6 ± 16.1 | 1 |
| **$GL_{proximal}$ (m/s)[a]** | 7.4 ± 2 | 7.0 ± 1.9 | -3.9 ± 22.6 | 1 | 7.9 ± 1.8 | 6.7 ± 2.1 | -13.8 ± 22.17 | 0.03 | 8.7 ± 1.8 | 9.5 ± 2.2 | 11.8 ± 21.0 | 1 |
| **$GL_{intermediate}$ (m/s)[a]** | 7.8 ± 2.1 | 7.2 ± 2.1 | -5.9 ± 16.5 | 1 | 8.3 ± 1.9 | 7.5 ± 2.2 | -10.4 ± 16.1 | 1 | 9.2 ± 2.0 | 9.4 ± 1.8 | 3.0 ± 17.0 | 1 |
| **$GL_{distal}$ (m/s)[a]** | 8.6 ± 1.9 | 8.1 ± 1.9 | -5.9 ± 14.3 | 1 | 9.0 ± 2.0 | 7.9 ± 2.0 | -11.2 ± 14.8 | 0.22 | 9.8 ± 2.1 | 10.0 ± 2.2 | 3.6 ± 0.3 | 1 |
| **$SOL_{proximal}$ (m/s)[a]** | 3.6 ± 1.1 | 3.0 ± 0.8 | -13.9 ± 19.3 | 1 | 3.9 ± 0.9 | 3.6 ± 0.8 | -6.4 ± 16.9 | 1 | 4.1 ± 1.4 | 4.1 ± 1.1 | 1.9 ± 23.0 | 1 |
| **$SOL_{distal}$ (m/s)[a]** | 3.8 ± 0.9 | 3.8 ± 1.1 | 3.9 ± 24.9 | 1 | 4.4 ± 1.0 | 3.8 ± 0.8 | -10.2 ± 22.8 | 1 | 4.5 ± 1.3 | 4.7 ± 1.1 | 12.0 ± 41.0 | 1 |
| **Sciatic shear wave velocity** | | | | | | | | | | | | |
| **$Sciatic_{proximal}$ (m/s)[b]** | 6.1 ± 1.5 | 4.9 ± 1.3 | -18.2 ± 15.7 | - | 6.2 ± 0.8 | 6.4 ± 0.8 | 3.7 ± 10.8 | - | 6.7 ± 2 | 7.1 ± 1.9 | 8.4 ± 13.1 | - |
| **$Sciatic_{distal}$ (m/s)[b]** | 7.2 ± 1.7 | 5.3 ± 1.5 | -25.3 ± 14.3 | - | 7.2 ± 1.2 | 7.3 ± 1.1 | 2.5 ± 14.7 | - | 8.1 ± 1.4 | 8.4 ± 1.5 | 3.8 ± 8.5 | - |
| **Tibial (m/s)[b]** | 7.3 ± 1.4 | 6.4 ± 1.3 | -13.7 ± 13.5 | - | 7.7 ± 1.6 | 8.0 ± 1.5 | 5.4 ± 16.6 | - | 9.0 ± 1.5 | 9.5 ± 1.2 | 6.3 ± 9.7 | - |

Values are mean ± SD. Legend: GM, *gastrocnemius medialis*; SOL, soleus.
**Muscle shear wave velocity:** **[a]** P-value $_{\text{interaction group × muscle × time}} = 0.035$
**Nerve shear wave velocity:** **[b]** P-value $_{\text{interaction group × nerve region × time}} = 0.587$; **[c]** P-value $_{\text{interaction group × time}} < 0.0001$

*Sciatic nerve shear wave velocity*

There was no group × nerve region × time main interaction effect (P = 0.587) for the shear wave velocity at maximum dorsiflexion ROM of pre-testing (Table 2). Also, neither region × time (P = 0.065) interaction nor group × nerve region (P = 0.148) were observed. However, a significant group × time effect was found (P < 0.0001; $_{p}\eta^{2}$ = 0.66) suggesting that mechanical adaptations to stretch are homogeneous through the nerve tract (Figure 5). *Post hoc* tests revealed a decrease in shear wave velocity in sciatic STR group after training (-19.1±15.3%; P < 0.0001), whereas no changes were observed in muscle STR (3.9±14.2%; P = 1) and CON groups (6.4±11.1%; P = 0.076).

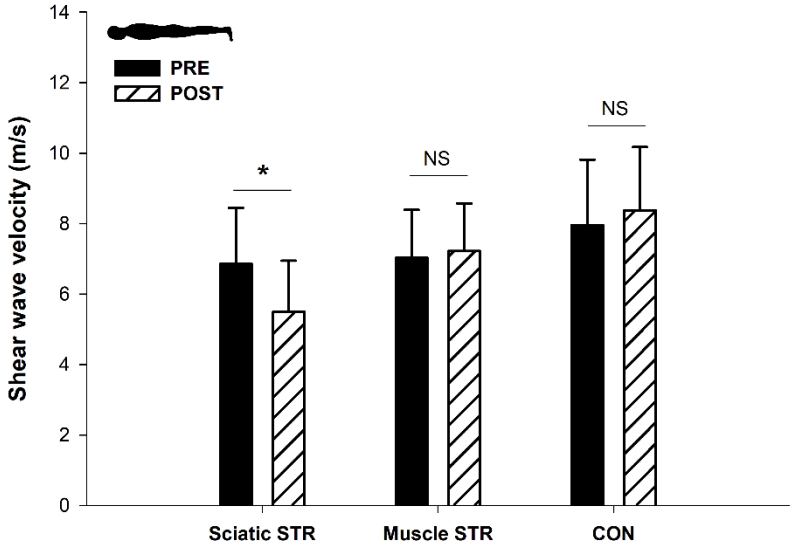



Figure 5. The effects of stretch interventions on local sciatic nerve shear wave velocity. The observed group × time effect (P < 0.0001) suggests that mechanical adaptations to stretch are homogeneous through the nerve course. *Post hoc* tests revealed a pre- to post-training decrease in shear wave velocity in sciatic STR group ($^{*}$P < 0.0001), and no changes occurred in both muscle STR ($^{NS}$P = 1) and CON groups ($^{NS}$P = 0.076).

A significant negative correlation (r = -0.58, P = 0.0007) was observed between the decrease in $SCI_{distal}$ shear wave velocity following the sciatic STR and the increase in maximal ROM in dorsiflexion (°) assessed in HIP-flexed position (Figure 6).

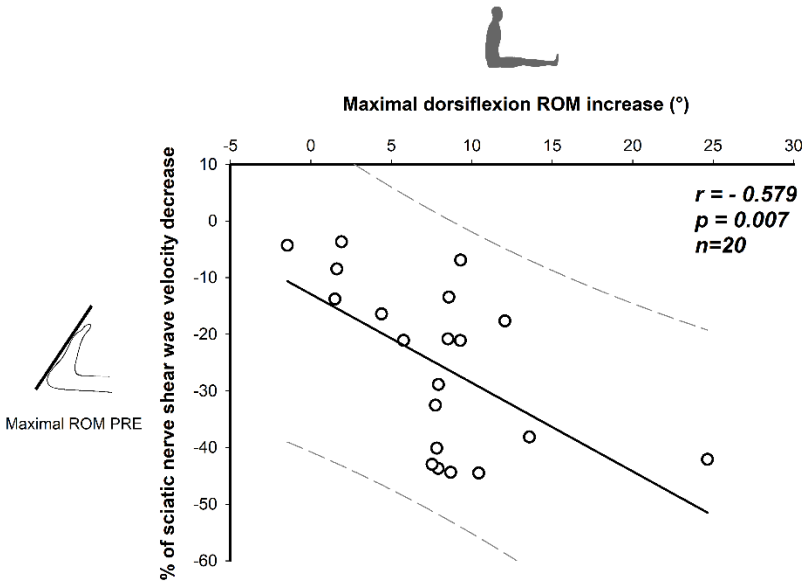



Figure 6. The effects of sciatic STR on local sciatic nerve stiffness (i.e. $sciatic_{distal}$) and maximal ankle ROM in dorsiflexion. The negative correlation coefficient shows that the sciatic STR induced an increase in maximal ankle ROM in dorsiflexion (°) measured in HIP-flexed position is correlated with the percentage decrease in sciatic nerve stiffness.

*Ankle passive torque*

A significant group × time (P = 0.004; $_p\eta^2$ = 0.18) interaction was observed in the pre-testing ankle torque assessed at maximum dorsiflexion ROM in HIP-neutral (Figure 7). There was a pre- to post-training decrease in passive ankle torque for the muscle STR group (-4.1±6.7 N.m; P = 0.03). No changes were found in passive torque after the training period in sciatic STR (-1.2±6.5 N.m; P = 1) and CON groups (3.0±5.6 N.m; P = 1). When assessed in HIP-flexed, there was neither significant group × time interaction effect (P = 0.56; $_p\eta^2$ = 0.02) nor main effects of time (P = 0.06; $_p\eta^2$ = 0.06) on ankle passive torque at maximum dorsiflexion ROM.

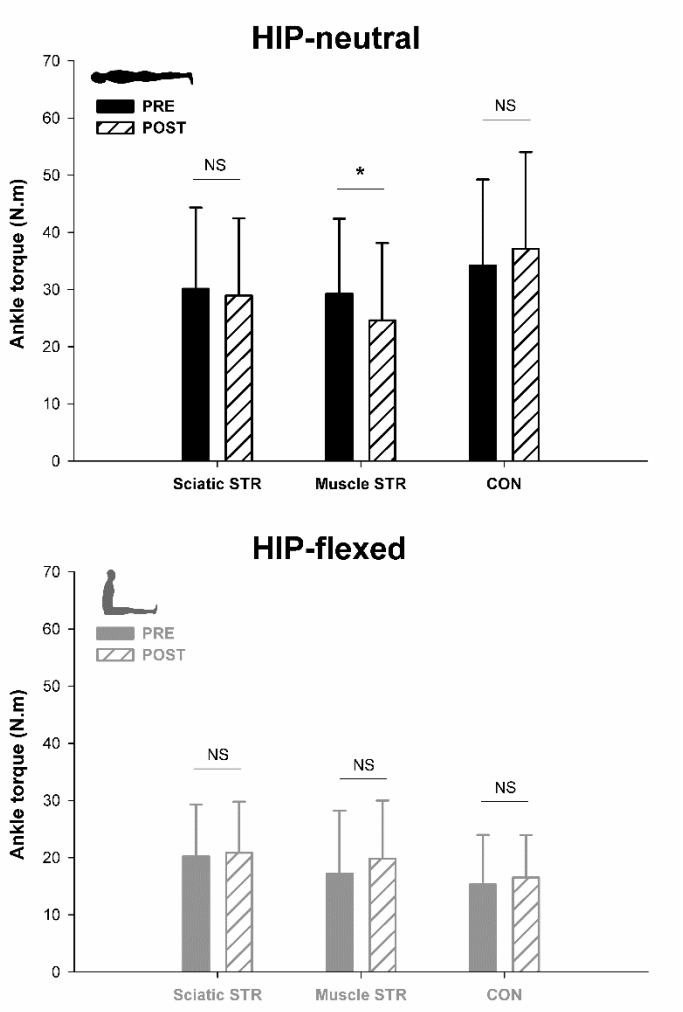



Figure 6. The effects of stretch training on ankle passive torque at a given angle. For the HIP-neutral testing position significant group × time interaction (P = 0.004) followed by *post hoc* analysis demonstrates a pre- to post-training decrease in passive ankle torque in muscle STR ($^{*}$P = 0.03), while no changes are observed in sciatic STR ($^{NS}$P = 1) and CON ($^{NS}$P = 1) groups. For the HIP-flexed no significant changes are observed on passive ankle torque significant group × time interaction effect (P = 0.56).

*RMS EMG*

Neither significant group × muscle × time, nor group × time interactions or time effect were observed either for the mean RMS EMG on the whole stretching cycle (P > 0.098, $_{p}\eta^{2}$ = 0.08) or RMS EMG assessed at maximal dorsiflexion ROM (P > 0.26, $_{p}\eta^{2}$ = 0.05) (Table 3).

Table 3. Pre and post-training values for each stretch group of RMS EMG measured in GM and SOL muscles at maximal dorsiflexion ROM and averaged through dorsiflexion range.

| | Sciatic STR | | Muscle STR | | CON | |
|---|---|---|---|---|---|---|
| Muscle | **PRE** | **POST** | **PRE** | **POST** | **PRE** | **POST** |
| **Averaged RMS EMG** | | | | | | |
| GM (%) | 0.8 ± 0.8 | 0.6 ± 0.4 | 0.4 ± 0.2 | 0.6 ± 0.6 | 0.4 ± 0.2 | 0.5 ± 0.2 |
| SOL (%) | 0.9 ± 1 | 0.6 ± 0.5 | 0.6 ± 0.5 | 0.8 ± 0.9 | 0.4 ± 0.2 | 0.6 ± 0.4 |
| **EMG at maximal dorsiflexion ROM** | | | | | | |
| GM (%) | 1.4 ± 2.3 | 1.1 ± 2.4 | 1.1 ± 1.7 | 0.8 ± 1 | 0.9 ± 1.2 | 0.9 ± 1.3 |
| SOL (%) | 1.7 ± 3.7 | 1.4 ± 3.5 | 1.9 ± 3.2 | 1.1 ± 1.4 | 0.9 ± 1.2 | 0.9 ± 1.2 |

Legend: GM, *gastrocnemius medialis*; SOL, soleus; ROM: range of motion; RMS: root mean square; EMG: electrography.

## DISCUSSION

The present study demonstrates that both the muscle and nerve stretching training protocols were effective at increasing the ankle dorsiflexion ROM, with mechanical adaptations specific to the targeted tissues. Specifically, a significant decrease in both plantar flexor stiffness and ankle passive torque was observed in the muscle STR group, while the sciatic nerve stiffness remained unaltered. In contrast, a decrease in sciatic nerve stiffness was observed in the sciatic STR group, in the absence of any change in muscle stiffness and ankle passive torque.

The present observation that chronic stretching increases the maximal ankle dorsiflexion ROM is in accordance with the vast majority of previous works (Guissard & Duchateau, 2004; Nakamura *et al.*, 2012; Akagi & Takahashi, 2014; Blazevich *et al.*, 2014). Interestingly, stretch training of plantar flexors muscles (muscle STR) and sciatic nerve (sciatic STR) induced significant increases in ankle ROM assessed in HIP-neutral and HIP-flexed positions, respectively. This confirms that that muscular and non-muscular structures should be responsible to the limitation in maximal dorsiflexion ROM in the HIP-neutral and HIP-flexed positions, respectively (Andrade *et al.*, 2016a; Nordez *et al.*, 2017). However, one important and novel finding is that the maximal joint ROM can also be chronically improved in HIP-neutral, in which ROM was thought to be mostly

limited by muscle-tendon units, by altering the mechanical properties of non-muscular structures (i.e. sciatic nerve).

Our results show that the effects of long-term stretch training on ankle flexibility are structure-dependent. Previous research has indicated that it is possible to selectively load muscular and non-muscular structures acting at the ankle joint by manipulating the position of both the hip and the knee joint (Cruz-Montecinos *et al.*, 2015; Andrade *et al.*, 2016a; Le Sant *et al.*, 2017). Specifically, hip flexion (HIP-flexed, knee fully extended) may limit significantly the maximal ankle ROM in dorsiflexion, being this traditionally attributed to the stretching of sciatic nerve (Mitchell *et al.*, 2008; Andrade *et al.*, 2016a). However, when tested in HIP-neutral position (knee fully-extended), the maximal ROM in dorsiflexion should be limited by the passive resistive to stretch of plantar flexors muscles (Gajdosik, 2001; Weppler & Magnusson, 2010; Nordez *et al.*, 2017). Accordingly, muscle STR did not induce changes in ankle ROM in HIP-flexed position, which confirms that mechanical properties of plantar flexors play a trivial role on the limitation of maximal dorsiflexion ROM in HIP-flexed position. As expected, ankle ROM in dorsiflexion did not differ between pre- and post-training (i.e. HIP-flexed and HIP-neutral testing positions) in CON group.

As most of the chronic stretching studies reported improvements in maximal ROM in the absence of mechanical adaptions at both joint and muscle-tendon tissues levels, the sensory theory remained the most plausible theory to explain the chronic effects of stretching (Weppler & Magnusson, 2010; Freitas *et al.*, 2017). The present results demonstrates that a longer muscle stretching protocol (i.e. 12 weeks) than those proposed in the literature is efficient to trigger significant adaptations in muscle mechanical properties and, as a result, in joint passive torque (Table 2, Figure 4 and Figure 6). Therefore, factors such as higher stretch volume and maximal stretch intensity were decisive for such mechanical adaptations in both skeletal muscles and joint torque.

We observed a decrease in ankle passive torque that reflects the stiffness changes of all muscle-tendon units acting across the joint (Magnusson, 1998; Gajdosik, 2001). Elastography measurements revealed that only some muscle locations exhibited significantly changes. Although there was a tendency to observe a global reduction of $GM_{proximal}$, $GL_{intermediate}$, $GL_{distal}$ and SOL passive muscle stiffness (stiffness decreased between 6.4% and 15.2%), statistically significant

mechanical changes were only found in $GM_{intermediate}$ (-14.3%), $GM_{distal}$ (-15.2%) and $GL_{proximal}$ (-13.8%). Therefore, the decrease in passive torque after muscle STR is not representative of the entire *triceps surae* because they did not display homogeneous between and within muscle adaptations. This strengthens the interest of use localized shear wave velocity (directly linked to the shear modulus) measurements to classical passive torque measurements in order to analyze the chronic effects of stretching. Akagi and Takahashi (2014) found a decrease of muscle transverse stiffness (reported as hardness) in both GM and GL after 5-weeks of *plantar flexors* stretching. However, both transverse scanning and the ankle angle (30° of plantar flexion, before the slack angle of all *gastrocnemii muscles*) that were adopted to perform the measurements were not appropriate (Gennisson et al., 2010). Interestingly, though, they did not find changes in ankle passive torque for a given angle. Thus, a novel and important finding of the present study is that stiffness adapts in a non-uniformly within the skeletal muscle following a chronic stretching protocol.

Although our muscle STR protocol was designed to specifically target both bi- and -mono articular plantar flexors (see methods), we did not observe mechanical adaptations in SOL muscle. Interestingly, Hirata *et al.* (2016) have reported similar results for after an acute intervention. Indeed, SOL, GM and GL muscles account on average for about 52%, 32% and 16% of the total *triceps surae* volume, respectively (Albracht *et al.*, 2008). Thus, it is possible that both stretch intensity and training duration may not have been sufficient to trigger stiffness adaptations within bulky SOL muscle as it was observed in local sites of GM and GL muscles.

Stiffness adaptations can be explained at two main levels: mechanical and physiological. Mechanically, changes in slack length such as a shift to the right of the passive stiffness-angle relationship (demonstrated following an acute stretching by Hirata *et al.* (2017)), and/or changes in intrinsic muscle elasticity could have contributed to the decrease in passive muscle stiffness. At a physiological level, histological changes in connective tissue and/or an increase in fiber length could explain the decrease in muscle stiffness. **Architecture currently being processed can contribute significantly to better know the origin of such stiffness adaptations.**

A decrease in sciatic nerve stiffness was observed following the sciatic STR, suggesting that adaptations in mechanical properties of non-muscular structures can explain the increase in ankle

ROM when they are mechanically stressed (e.g. HIP-flexed for the sciatic nerve). Hence, this study provides the first *in-vivo* experimental evidence that peripheral nerves can adapt mechanically to long-term stretch stimuli. Localized variation in collagen fibril diameter has been suggested to contribute to the longitudinal heterogeneity of tensile properties in this nerve (Mason & Phillips, 2011). Previous findings have demonstrated that strain on nerves during limb movement is not equally distributed along their length, but is increased close to joints (Phillips et al., 2004). Although we observed heterogeneous distribution of stiffness along the sciatic nerve tract (shear wave velocity $sciatic_{proximal} < sciatic_{distal} <$ tibial), the nerve stiffness adaptations were independent of the anatomical region where measurements were performed. Such homogeneous adaptations, in spite of spatial variations, may be linked to the biomechanically complexity of nerves during joint(s) motion(s) (Topp & Boyd, 2006; Silva *et al.*, 2014). Future research should investigate mechanical behavior of nerves and their adaptations to loading in joint and non-joint regions. In addition, we speculate that nerve stiffness adaptations are related to the dense connective tissue network present along the nerve length. Indeed, most of the mechanical protection of peripheral nerves is provided by the epineurium, perineurium and endoneurium. While it has been suggested that wavy collagen arrangement (Type I and Type III) and elastic fibers that comprise the epineurium provides mechanical protection from compression (Stolinski, 1995; Topp & Boyd, 2006), the perineurium has been considered as the primary contributor to the nerve's viscoelastic properties (Rydevik et al., 1990; Sunderland, 1990; Topp & Boyd, 2006). Thus, changes in the collagen content due to stretch stimuli (Kjaer, 2004) could have contributed to nerve stiffness decrease.

The present study shows that it is possible to selectively trigger mechanical adaptations in nervous system without significantly stress the MTU(s) acting on the target joint, and vice-versa. Indeed, neurodynamic testing has traditionally been used in clinical practice to assess and manipulate the mobility and integrity of the peripheral nervous system (Butler, 1991; Shacklock, 2005). Briefly, nerves can be tensioned mechanically while they are elongated in a longitudinal direction by combining movements of one or several joints that induce displacement of the nerve endings in opposite directions. Thus, the results of the present study are of main importance to orthopedic and physical therapy fields because they may help in design and prescription of individualized stretching programs, and to allow a more individual interpretation of the clinical value of loading muscular and non-muscular structures during maximal ROM assessments. Such

programs could be particularly beneficial in some neurological and musculoskeletal conditions that induce mechanical dysfunctions on nerves such as increased local nerve stiffness (Kantarci *et al.*, 2014; Dikici *et al.*, 2016; Ishibashi *et al.*, 2016) which may be responsible for losses in maximal joint ROM.

**Conclusion**

The results of this study suggests that: i) muscle and nerve stiffness can be selectively decreased with a specific chronic stretching intervention, and consequently increase the maximal joint ROM; and ii) while muscle stretching only alters the maximal joint ROM for the body posture used during the intervention (HIP-neutral), the nerve stretching increases the maximal joint ROM in different body postures (i.e., HIP-flexed and HIP-neutral).

## REFERENCES

Akagi R & Takahashi H. (2013). Acute effect of static stretching on hardness of the gastrocnemius muscle. *Medicine and science in sports and exercise* **45,** 1348-1354.

Akagi R & Takahashi H. (2014). Effect of a 5-week static stretching program on hardness of the gastrocnemius muscle. *Scandinavian journal of medicine & science in sports* **24,** 950-957.

Albracht K, Arampatzis A & Baltzopoulos V. (2008). Assessment of muscle volume and physiological cross-sectional area of the human *triceps surae* muscle in vivo. *Journal of biomechanics* **41,** 2211-2218.

Andrade RJ, Lacourpaille L, Freitas SR, McNair PJ & Nordez A. (2016a). Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension. *Scandinavian journal of medicine & science in sports* **26,** 41-47.

Andrade RJ, Nordez A, Hug F, Ates F, Coppieters MW, Pezarat-Correia P & Freitas SR. (2016b). Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography. *Journal of biomechanics* **49,** 326-331.

Bercoff J, Tanter M & Fink M. (2004). Supersonic shear imaging: a new technique for soft tissue elasticity mapping. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **51,** 396-409.

Blazevich AJ, Cannavan D, Waugh CM, Miller SC, Thorlund JB, Aagaard P & Kay AD. (2014). Range of motion, neuromechanical, and architectural adaptations to plantar flexor stretch training in humans. *Journal of applied physiology* **117,** 452-462.

Brum J, Bernal M, Gennisson JL & Tanter M. (2014). In vivo evaluation of the elastic anisotropy of the human Achilles tendon using shear wave dispersion analysis. *Physics in medicine and biology* **59,** 505-523.

Butler DS. (1991). *Mobilisation of the Nervous System*. Churchill Livingstone.

Cohen J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J. : L. Erlbaum Associates.

Cruz-Montecinos C, Gonzalez Blanche A, Lopez Sanchez D, Cerda M, Sanzana-Cuche R & Cuesta-Vargas A. (2015). In vivo relationship between pelvis motion and deep fascia displacement of the medial gastrocnemius: anatomical and functional implications. *Journal of anatomy* **227,** 665-672.

DeWall RJ, Slane LC, Lee KS & Thelen DG. (2014). Spatial variations in Achilles tendon shear wave speed. *Journal of biomechanics* **47,** 2685-2692.

Dikici AS, Ustabasioglu FE, Delil S, Nalbantoglu M, Korkmaz B, Bakan S, Kula O, Uzun N, Mihmanli I & Kantarci F. (2016). Evaluation of the Tibial Nerve with Shear-Wave Elastography: A Potential Sonographic Method for the Diagnosis of Diabetic Peripheral Neuropathy. *Radiology***,** 160135.

Ekstrand J, Gillquist J & Liljedahl SO. (1983). Prevention of soccer injuries. Supervision by doctor and physiotherapist. *The American journal of sports medicine* **11,** 116-120.

Freitas SR, Mendes B, Le Sant G, Andrade RJ, Nordez A & Milanovic Z. (2017). Can chronic stretching change the muscle-tendon mechanical properties? A review. *Scandinavian journal of medicine & science in sports*.

Gajdosik RL. (2001). Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clinical biomechanics* **16,** 87-101.

Gennisson JL, Deffieux T, Mace E, Montaldo G, Fink M & Tanter M. (2010). Viscoelastic and anisotropic mechanical properties of in vivo muscle tissue assessed by supersonic shear imaging. *Ultrasound in medicine & biology* **36,** 789-801.

Guissard N & Duchateau J. (2004). Effect of static stretch training on neural and mechanical properties of the human plantar-flexor muscles. *Muscle & nerve* **29,** 248-255.

Harvey LA & Herbert RD. (2002). Muscle stretching for treatment and prevention of contracture in people with spinal cord injury. *Spinal cord* **40,** 1-9.

Helfenstein-Didier C, Andrade RJ, Brum J, Hug F, Tanter M, Nordez A & Gennisson JL. (2016). In vivo quantification of the shear modulus of the human Achilles tendon during passive loading using shear wave dispersion analysis. *Physics in medicine and biology* **61,** 2485-2496.

Hermens HJ, Freriks B, Disselhorst-Klug C & Rau G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology* **10,** 361-374.

Hirata K, Kanehisa H & Miyamoto N. (2017). Acute effect of static stretching on passive stiffness of the human gastrocnemius fascicle measured by ultrasound shear wave elastography. *European journal of applied physiology* **117,** 493-499.

Hirata K, Miyamoto-Mikami E, Kanehisa H & Miyamoto N. (2016). Muscle-specific acute changes in passive stiffness of human *triceps surae* after stretching. *European journal of applied physiology* **116,** 911-918.

Ishibashi F, Taniguchi M, Kojima R, Kawasaki A, Kosaka A & Uetake H. (2016). Elasticity of the tibial nerve assessed by sonoelastography was reduced before the development of

neuropathy and further deterioration associated with the severity of neuropathy in patients with type 2 diabetes. *Journal of diabetes investigation* **7,** 404-412.

Kantarci F, Ustabasioglu FE, Delil S, Olgun DC, Korkmazer B, Dikici AS, Tutar O, Nalbantoglu M, Uzun N & Mihmanli I. (2014). Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. *Eur Radiol* **24,** 434-440.

Kjaer M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological reviews* **84,** 649-698.

Le Sant G, Nordez A, Andrade R, Hug F, Freitas S & Gross R. (2017). Stiffness mapping of lower leg muscles during passive dorsiflexion. *Journal of anatomy* **230,** 639-650.

Magnusson SP. (1998). Passive properties of human skeletal muscle during stretch maneuvers. A review. *Scandinavian journal of medicine & science in sports* **8,** 65-77.

Mason S & Phillips JB. (2011). An ultrastructural and biochemical analysis of collagen in rat peripheral nerves: the relationship between fibril diameter and mechanical properties. *Journal of the peripheral nervous system : JPNS* **16,** 261-269.

Mitchell B, Bressel E, McNair PJ & Bressel ME. (2008). Effect of pelvic, hip, and knee position on ankle joint range of motion. *Phys Ther Sport* **9,** 202-208.

Nakamura M, Ikezoe T, Takeno Y & Ichihashi N. (2012). Effects of a 4-week static stretch training program on passive stiffness of human gastrocnemius muscle-tendon unit in vivo. *European journal of applied physiology* **112,** 2749-2755.

Neto T, Freitas SR, Marques M, Gomes L, Andrade R & Oliveira R. (2017). Effects of lower body quadrant neural mobilization in healthy and low back pain populations: A systematic review and meta-analysis. *Musculoskeletal Science and Practice* **27,** 14-22.

Nordez A, Gross R, Andrade RJ, Le Sant G, Freitas SR, Ellis R, McNair PJ & Hug F. (2017). Non-Muscular Structures Can Limit the Maximal Joint Range of Motion during Stretching. *Sports medicine* **[Epub ahead of print]**.

Nordez A, McNair P, Casari P & Cornu C. (2008). Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. *International journal of sports medicine* **29,** 414-418.

Phillips JB, Smit X, De Zoysa N, Afoke A & Brown RA. (2004). Peripheral nerves in the rat exhibit localized heterogeneity of tensile properties during limb movement. *The Journal of physiology* **557,** 879-887.

Pin T, Dyke P & Chan M. (2006). The effectiveness of passive stretching in children with cerebral palsy. *Developmental medicine and child neurology* **48,** 855-862.

Randhawa A, Jackman ME & Wakeling JM. (2013). Muscle gearing during isotonic and isokinetic movements in the ankle plantarflexors. *European journal of applied physiology* **113,** 437-447.

Rydevik BL, Kwan MK, Myers RR, Brown RA, Triggs KJ, Woo SL & Garfin SR. (1990). An in vitro mechanical and histological study of acute stretching on rabbit tibial nerve. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **8,** 694-701.

Shacklock M. (2005). *Clinical Neurodynamics: A New System of Musculoskeletal Treatment*. Elsevier Butterworth-Heinemann.

Shah SB. (2017). Tissue Biomechanics: Whales Have Some Nerve. *Curr Biol* **27,** R177-R179.

Silva A, Manso A, Andrade R, Domingues V, Brandao MP & Silva AG. (2014). Quantitative in vivo longitudinal nerve excursion and strain in response to joint movement: A systematic literature review. *Clinical biomechanics* **29,** 839-847.

Stolinski C. (1995). Structure and composition of the outer connective tissue sheaths of peripheral nerve. *Journal of anatomy* **186 ( Pt 1),** 123-130.

Sunderland S. (1990). The anatomy and physiology of nerve injury. *Muscle & nerve* **13,** 771-784.

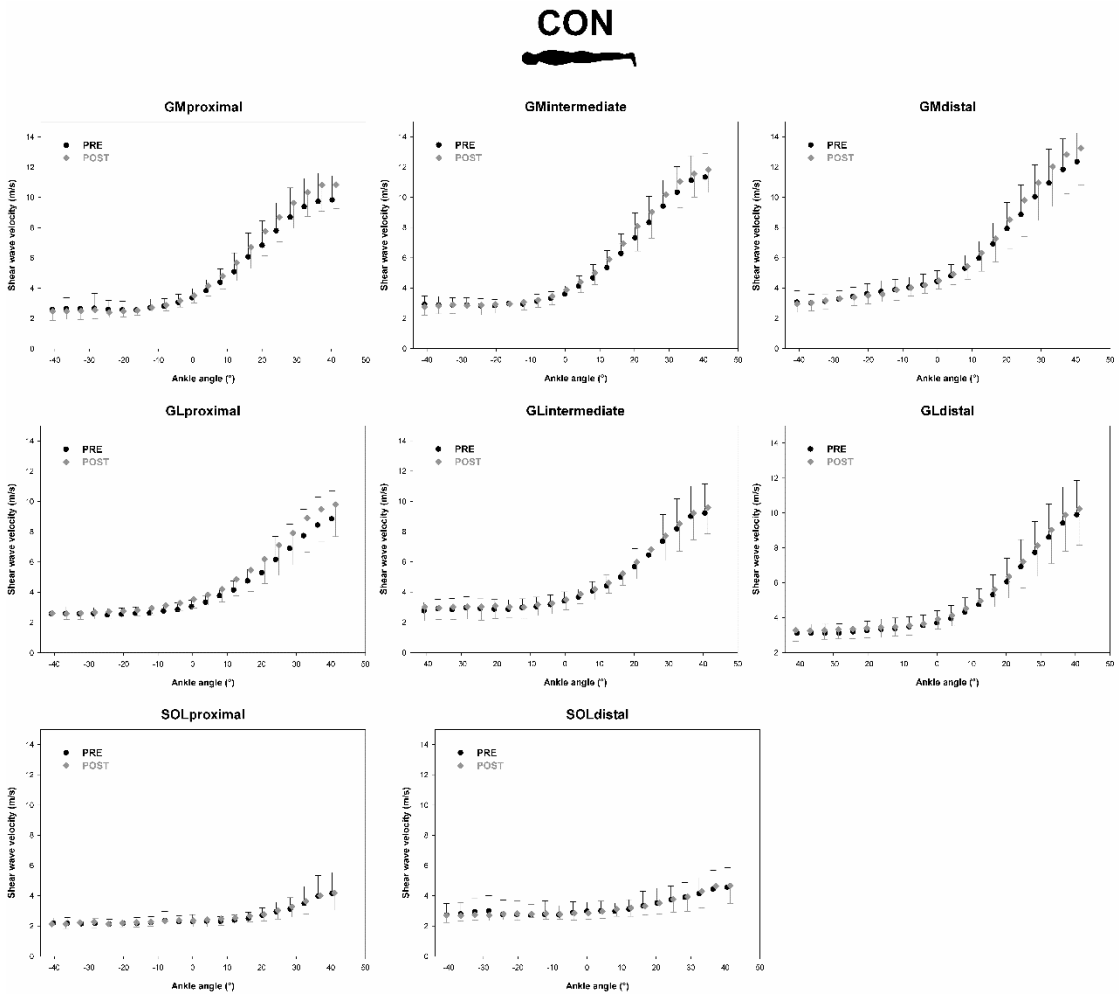
Topp KS & Boyd BS. (2006). Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Physical therapy* **86,** 92-109.

Weppler CH & Magnusson SP. (2010). Increasing muscle extensibility: a matter of increasing length or modifying sensation? *Physical therapy* **90,** 438-449.

Witvrouw E, Danneels L, Asselman P, D'Have T & Cambier D. (2003). Muscle flexibility as a risk factor for developing muscle injuries in male professional soccer players. A prospective study. *The American journal of sports medicine* **31,** 41-46.

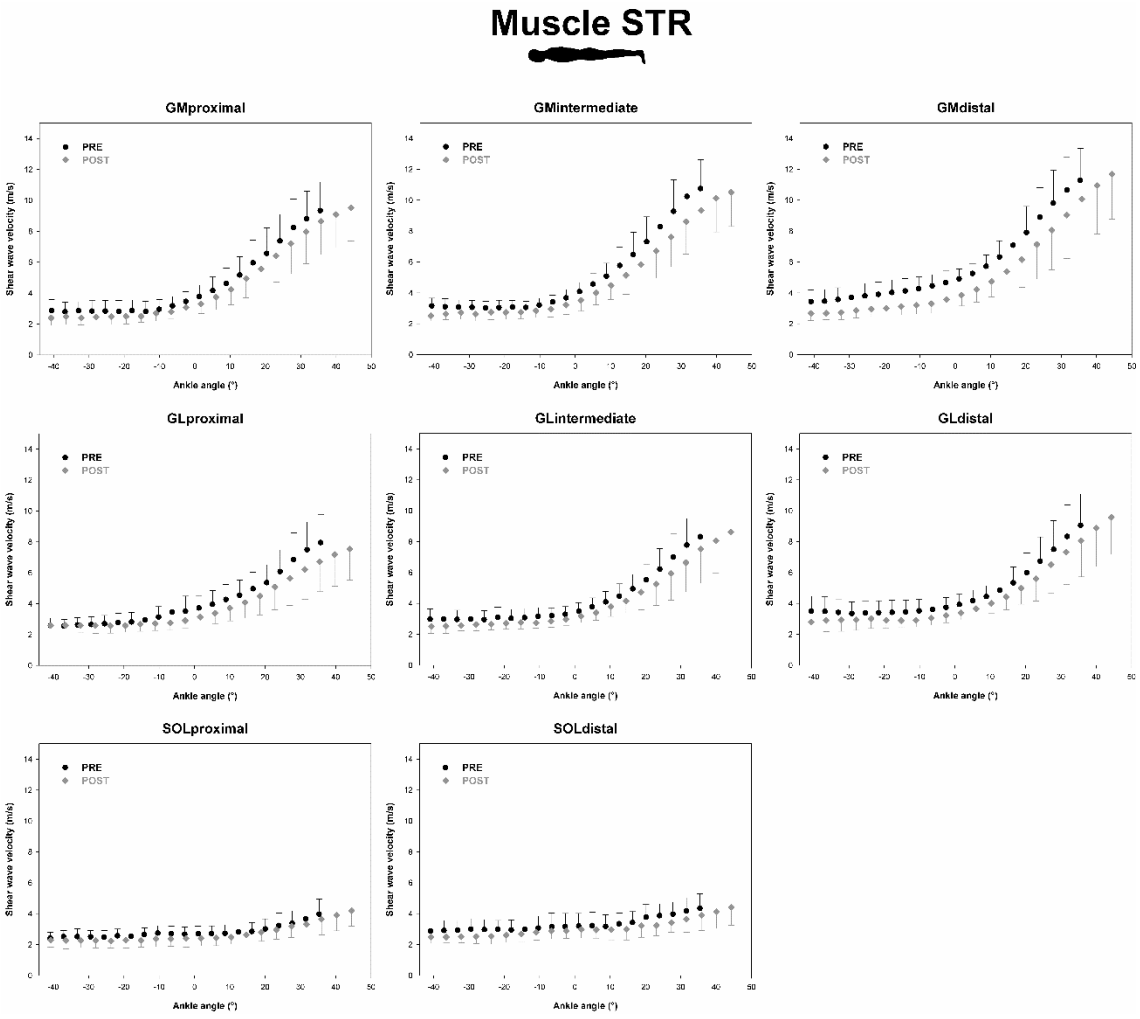
# [SUPPLEMENTARY DATA FILES]

## [FIGURE 1]



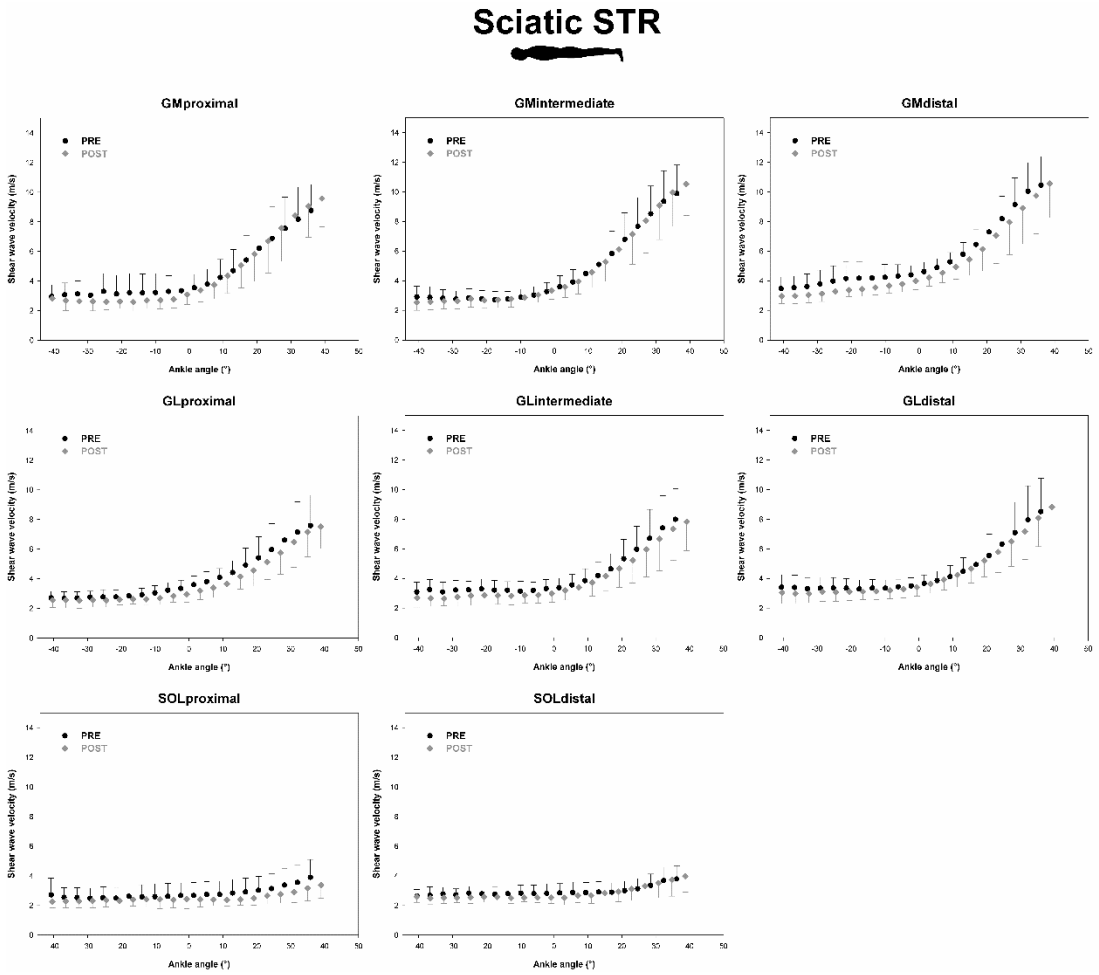

Relationships between the ankle angle and the *triceps surae* muscle shear wave velocity in CON group. Measurements were performed PRE and POST intervention in proximal, intermediate and distal parts of the gastrocnemii; and in proximal and distal for the soleus. GM (*gastrocnemius medialis*); GL (*gastrocnemius lateralis*); SOL (soleus). The ankle rotation started at -40° of plantar flexion. Positive ankle angles represent dorsiflexion ROM.

**[FIGURE 2]**



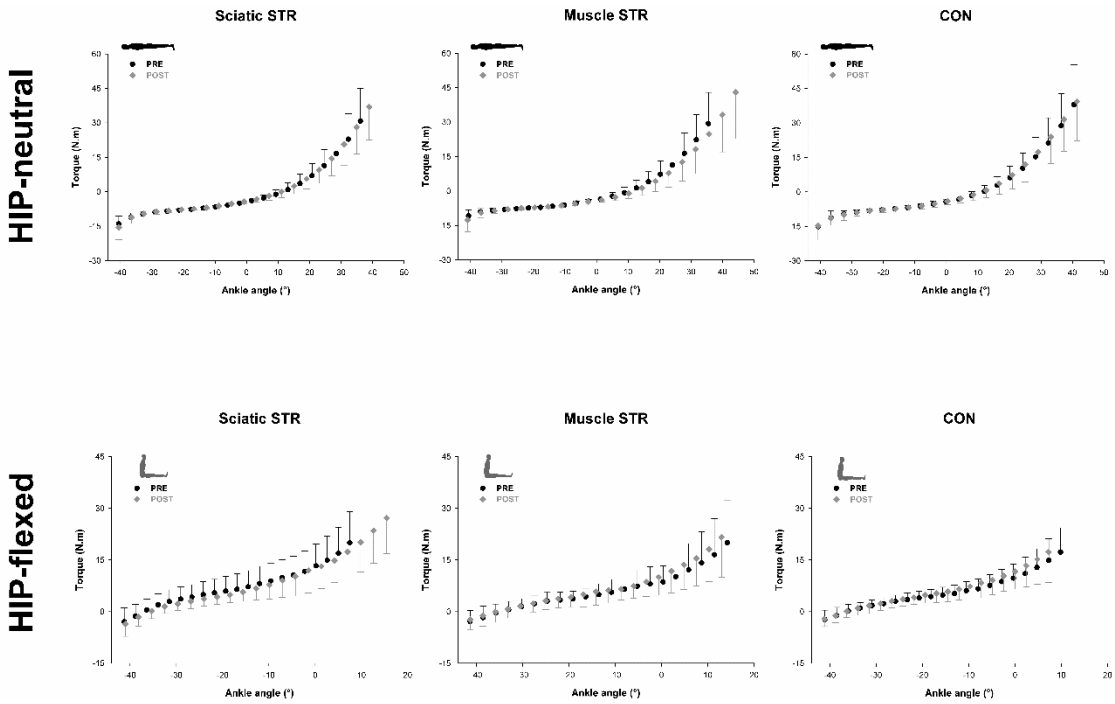

Relationships between the ankle angle and the *triceps surae* muscle shear wave velocity in muscle STR group. Measurements were performed PRE and POST intervention in proximal, intermediate and distal parts of the gastrocnemii; and in proximal and distal for the soleus. GM (*gastrocnemius medialis*); GL (*gastrocnemius lateralis*); SOL (soleus). The ankle rotation started at -40° of plantar flexion. Positive ankle angles represent dorsiflexion ROM.

# [FIGURE 3]



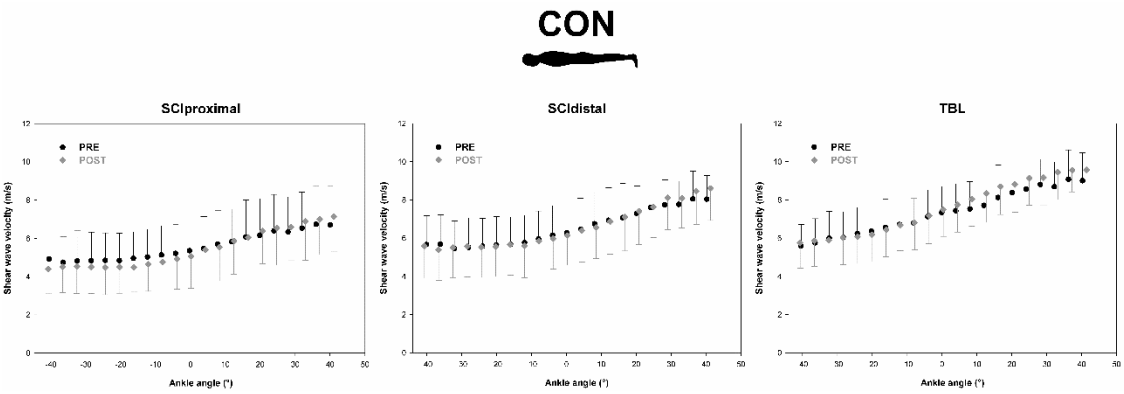

Relationships between the ankle angle and the *triceps surae* shear wave velocity in sciatic STR group. Measurements were performed PRE and POST intervention in proximal, intermediate and distal parts of the gastrocnemii; and in proximal and distal for the soleus. GM (*gastrocnemius medialis*); GL (*gastrocnemius lateralis*); SOL (soleus). The ankle rotation started at -40° of plantar flexion (negative values). Positive ankle angles represent dorsiflexion ROM.

**[FIGURE 4]**



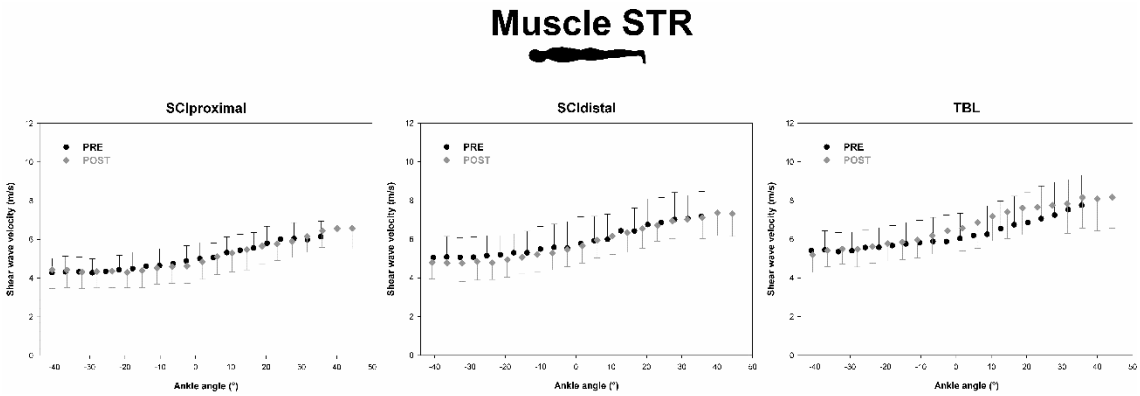

Relationships between the ankle angle and the passive torque for both HIP-neutral and HIP-flexed testing positions. Measurements were performed in sciatic STR, muscle STR and CON groups PRE and POST interventions. The ankle rotation started at -40° of plantar flexion (negative values). Positive ankle angles represent dorsiflexion ROM.

# [FIGURE 5]



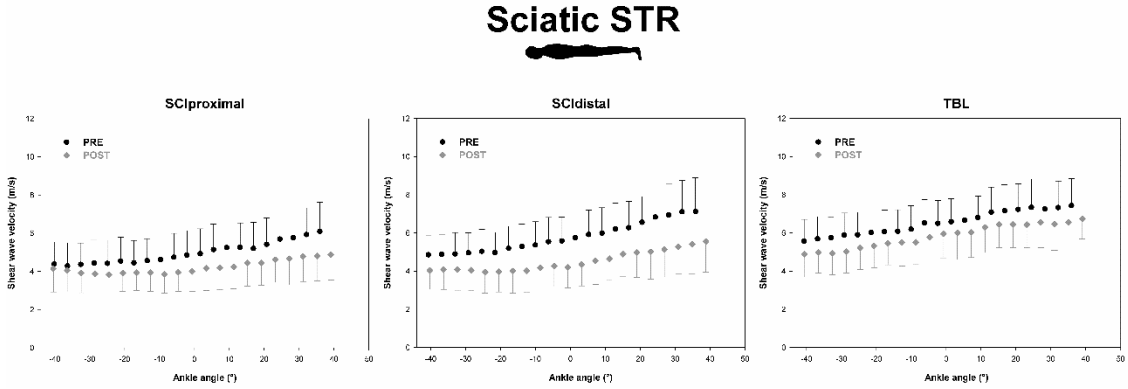

Relationships between the ankle angle and the sciatic nerve shear wave velocity in CON group. Measurements were performed PRE and POST intervention in proximal, and distal regions of sciatic nerve; and in tibial nerve immediately after the bifurcation point of sciatic nerve. SCI (sciatic nerve); TBL (tibial nerve). The ankle rotation started at -40° of plantar flexion. Positive ankle angles represent dorsiflexion ROM.

## [FIGURE 6]




Relationships between the ankle angle and the sciatic nerve shear wave velocity in muscle STR group. Measurements were performed PRE and POST intervention in proximal, and distal regions of sciatic nerve; and in tibial nerve immediately after the bifurcation point of sciatic nerve. SCI (sciatic nerve); TBL (tibial nerve). The ankle rotation started at -40° of plantar flexion. Positive ankle angles represent dorsiflexion ROM.

## [FIGURE 7]




Relationships between the ankle angle and the sciatic nerve shear wave velocity in sciatic STR group. Measurements were performed PRE and POST intervention in proximal, and distal regions of sciatic nerve; and in tibial nerve immediately after the bifurcation point of sciatic nerve. SCI (sciatic nerve); TBL (tibial nerve). The ankle rotation started at -40° of plantar flexion. Positive ankle angles represent dorsiflexion ROM

*Chapter Five*

# GENERAL DISCUSSION

This thesis aimed to extend the knowledge of the mechanical role of muscular and non-muscular tissues underpinning the maximal joint ROM at a given joint *in-vivo*. To this end, four studies were conducted to:

- See how joints and muscles respond mechanically to maximal joint ROM assessments at different postures (study 1);
- Explore whether mechanical properties of peripheral nerves can be assessed *in-vivo* during passive joint motion by using ultrasound shear wave elastography (study 2);
- Analyze whether it is possible to stretch and alter the stiffness of non-muscular structures in the absence of mechanical changes in muscles and if they are related with changes in maximal joint ROM (study 3);
- Determine if both acute and chronic stretching of either nerve or muscle tissues alter their mechanical properties and, thus, contribute to adaptations in maximal joint ROM (study 4).

The major finding of this doctoral research is that maximal joint ROM can be limited by the mechanical loading of either muscular or non-muscular structures, and is strongly dependent on the limb posture adopted for the assessments. Additionally, our results strongly suggest that the peripheral nerves can play an important role in the maximal joint ROM. Furthermore, this thesis provides direct evidence suggesting that appropriate stretch training can trigger mechanical adaptations in individual structures and, as a result, induce specific changes in maximal joint ROM.

The results from these studies are individually addressed in following three sections: 1) the influence of muscular and non-muscular structures in maximal ROM; 2) assessment of sciatic nerve stiffness non-invasively; and 3) tissue and joint mechanical adaptations to stretching.

## 1. Link between studies

Maximal dorsiflexion ROM was the common variable among the four studies. Study 1 showed that the maximal ankle ROM in dorsiflexion is affected notably when the hip is flexed from the neutral position to 90° (knee remains fully extended), in the absence of changes in local muscle stiffness (*gastrocnemius medialis*) and passive ankle torque. This result demonstrates directly that both stiffness of muscles acting on the joint and passive joint torque cannot explain the maximal

dorsiflexion ROM loss when changing the position of the hip. Therefore, it suggests that multi-articular and non-muscular structures that cross both the hip and ankle joints should be mechanically stressed during this combined positioning of lower limb joints.

Lower-limb fasciae and peripheral nerves seemed to be the best candidates to explain this effect. Indeed, cadaver findings have demonstrated the continuity of deep fasciae between both the thigh and the leg (Stecco & Hammer, 2015). However, assessment of the mechanical properties in this extensive network of connective tissue *in-vivo* still remained a big challenge for researchers mainly due to the tissues' small thickness. In contrast, the assessment of nerve stiffness using ultrasound shear wave elastography seemed less challenging and more obvious. In particular, the sciatic nerve is the thickest nerve of the human body, and some clinical tests such as straight leg raising and slump are often used to evaluate the mechanosensitivity of sciatic nerve system (including nerve roots) in the rehabilitation and orthopedic fields (Butler, 1991; Shacklock, 2005; Boyd et al., 2009). However, a direct assessment of nerve stiffness *in-vivo* was lacking.

Thus, in study 2 we showed that the stiffness of the sciatic nerve can be non-invasively assessed during passive ankle rotations (hip in neutral position) by using supersonic shear wave elastography. Interestingly, we also showed sciatic nerve loads during passive ankle dorsiflexion; but this is strongly dependent on the knee joint position. As such, this study confirmed the biomechanical complexity of the sciatic nerve in response to joint motion. Taking advantage of the findings of these two first studies, study 3 was designed to directly manipulate the sciatic nerve stiffness by changing the hip position and to examine the acute effects of decreased nerve stiffness on the maximal ROM. The findings of study 3 provide evidence that sciatic nerve stretching is efficient in improving the maximal dorsiflexion ROM, in particular when in lower limb postures where nerves and other non-muscular structures, such as lower limb *fasciae*, are maximally lengthened (HIP-flexed position). Interestingly, neither changes in ankle torque nor changes in local muscle stiffness were observed. Overall, these findings suggest that we can stretch peripheral nerves with minimal impact on muscle and joint stiffness acting on the target joint. However, influence of other non-muscular and continuous structures (e.g., fasciae) on the maximal ankle ROM in dorsiflexion are unable to be excluded by this study.

Concomitant with the observations from study 3, study 4 showed that improvements in maximal ROM following chronic stretching are specific to the hip testing position (HIP-neutral

and HIP-flexed) and stretching modalities: muscular vs. non-muscular stretching. Specifically, maximal ROM adaptations to chronic stretching are strongly dependent on the tissue and joint mechanical adaptations induced in target tissues.

## 2. Non-invasive assessment of sciatic nerve stiffness

Ultrasound shear wave elastography provides a significant opportunity to assess nerve mechanical properties in a non-invasive manner. At the time of its publication, study 2 was the second study using this technique to assess the mechanical properties of peripheral nerves. Indeed, Kantarci et al. (2014) had found high reproducibility of ultrasound shear wave elastography (SSI) measurements, but only in resting conditions and in the median nerve (upper limb). From a research perspective, it was necessary to explore whether the SSI could be a reliable measurement method to assess the mechanical properties of the sciatic nerve during joint motion.

Although there was intense pilot testing, we did not obtain reliable nerve stiffness measurements in the sciatic nerve for the HIP-flexed position. Our pilot tests suggested that the HIP-flexed position with the ankle at 40° in plantar flexion already significantly loads the sciatic nerve (for review: Pilot study). Moreover, because nerves are relatively stiff structures (for example, less stiff than tendons), shear waves propagate at a high velocity, making their measurement more challenging. Hence, measurements saturate at relatively low tension levels, e.g., about 20/10° in plantar flexion during passive stretching. Additional ankle rotation towards dorsiflexion will induce further nerve tension and higher saturation levels within the nerve structure. While these saturated measurements can be used to compare nerve tensile response across different body postures, as reported more recently by Greening and Dilley (2017), they do not allow to detect accurately either acute or chronic changes in nerve stiffness. Thus, adaptations in nerve stiffness to stretching were evaluated in the HIP-neutral (studies 3 and 4) rather than HIP-flexed position, where the nerve can be maximally stretched. As such, the important role of nerve stiffness in maximal dorsiflexion ROM should be considered as indirect evidence in this doctoral research.

Consequently, study 2 shows that sciatic shear wave velocity assessed using SSI provides a reliable measurement *in vivo* of sciatic nerve shear wave velocity during ankle rotations.

Specifically, the findings demonstrate a good repeatability of the sciatic shear wave velocity measured at the proximal third of the thigh during passive and slow ankle movements (SEM<0.34 m/s; CV<8.1%, and ICC>0.92). Results from this study highlight the potential of using shear wave elastography to non-invasively evaluate the mechanical properties of peripheral nerves at rest and during passive stretching. This doctoral research is the first to assess the reliability of measuring sciatic nerve shear wave velocity *in-vivo* during articular motion. It is also one of the first studies to attempt to non-invasively quantify the mechanical nerve loading of any of the peripheral nerves.

The sciatic nerve is thought to be a complex biomechanical system. From the findings of this thesis, in agreement with previous cadaver studies (Coppieters et al., 2006; Alshami et al., 2008; Boyd et al., 2013), the sciatic nerve should be mechanically loaded by the combination of specific articular positions of the spine, hip, knee, and ankle joints. As such, sciatic nerve tension might increase when is stretched due to specific positioning of multiple joints that the sciatic nerve crosses.

Study 2 showed that sciatic nerve stiffness increases at a proximal site with the ankle dorsiflexion, but this is only observed when the knee is fully-extended (i.e., 180°). This suggest that anatomical features of the sciatic nerve may explain the absence of stiffness changes during ankle rotation when the knee was flexed at 90º (HIP-neutral). Identically, in agreement with our pilot testing and findings from studies 3 and 4, Greening and Dilley (2017) have observed more recently that tibial nerve (sciatic nerve branch) stiffness increases significantly in postures that are known to lengthen the whole nerve tract. Both the tibial and sciatic nerve stiffness progressively increase from the HIP-neutral to the HIP-flexed position, with the knee angle being a key factor to transmitting mechanical forces within the sciatic nerve system between the thigh and leg during passive ankle rotations. Interestingly, our observations from study 4 suggest that the interpretation of our results should be confined to the specific location where the stiffness was measured. The sciatic nerve system displays a heterogeneous distribution of the stiffness across proximal and distal and at the tibial nerve at the level of popliteal fossa. In agreement with these findings, it was previously observed *in-vitro* that nerve architecture is different between joint and non-joint areas, and that the nerve regions near the joints undergo greater elongation than other regions during joint movement (Phillips et al., 2004).

# 3. The influence of muscular and non-muscular structures in maximal ROM

## 3.1. Individual contribution of nerve stiffness

While also considering the participant data from the pilot testing and studies 3 and 4 of the present research, we performed an additional analysis. The results of this additional analysis show a significant and positive correlation between the difference of maximal ROM assessed in both HIP angle testing (in degrees) and the sciatic nerve shear wave velocity (an index of stiffness). These correlations were found regardless the ankle angle (Table 2, Figure 29).

*Table 2. Correlations between the sciatic nerve shear wave velocity (at 20° in plantar flexion; neutral position of the ankle at 0°; and 10° and 20° in dorsiflexion) and the difference between maximum dorsiflexion ROM in HIP-flexed and HIP-neutral positions. Sciatic nerve shear wave velocity was assessed in the HIP-neutral position. This analysis included 87 subjects.*

| ***Ankle angle*** | ***R*** | ***P-value*** |
|---|---|---|
| *20° PF* | 0.575 | < 0.0001 |
| *0° N* | 0.580 | < 0.0001 |
| *10° DF* | 0.572 | < 0.0001 |
| *20° DF* | 0.561 | < 0.0001 |

*R: Pearson's correlation coefficient; PF: plantar flexion; DF: dorsiflexion; neutral position.*

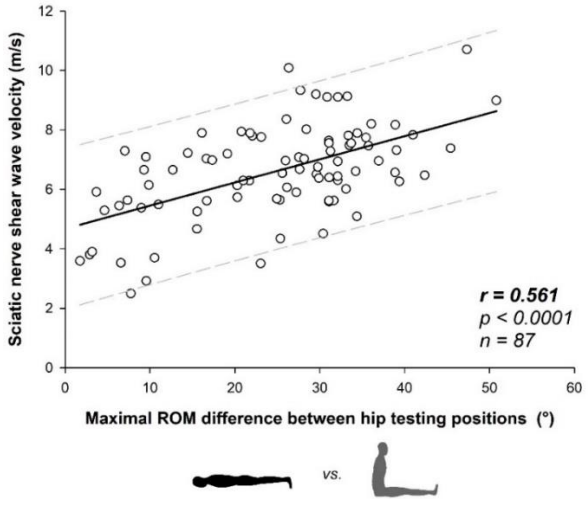



*Figure 29. Correlation between the sciatic nerve shear wave velocity (measured at 20° of ankle dorsiflexion) and the difference between maximum dorsiflexion ROM in the HIP-flexed and HIP-neutral positions. Sciatic nerve shear wave velocity was assessed in the HIP-neutral position.*

Importantly, the correlations demonstrate that a greater change in maximal ROM in dorsiflexion at the ankle joint was apparent when the hip position was changed from neutral to 90° flexion, and this was moderately correlated with higher stiffness in the sciatic nerve. Although no direct causality can be inferred from this correlation, this result strengthens the possibility that sciatic nerve stiffness may play a significant role on the maximal dorsiflexion ROM.

Concomitant with this finding, two interesting correlations were found in studies 3 and 4 that illustrated the role of the sciatic nerve on maximal ROM. Briefly, the increase in ankle dorsiflexion ROM assessed in the HIP-flexed position (i.e., a position that might considerably stretch the sciatic nerve tract) was moderately correlated with a decrease of sciatic nerve stiffness following either acute or chronic non-muscular stretching (Figure 30). This strongly suggests that the changes in mechanical properties of the sciatic nerve induced by the non-muscular stretching played an important role in the increase of maximal ROM. On the other hand, the correlations also indicate that most improvements in maximal ROM cannot be explained solely by the adaptations in nerve mechanical properties and, thus, still remain to be explored.

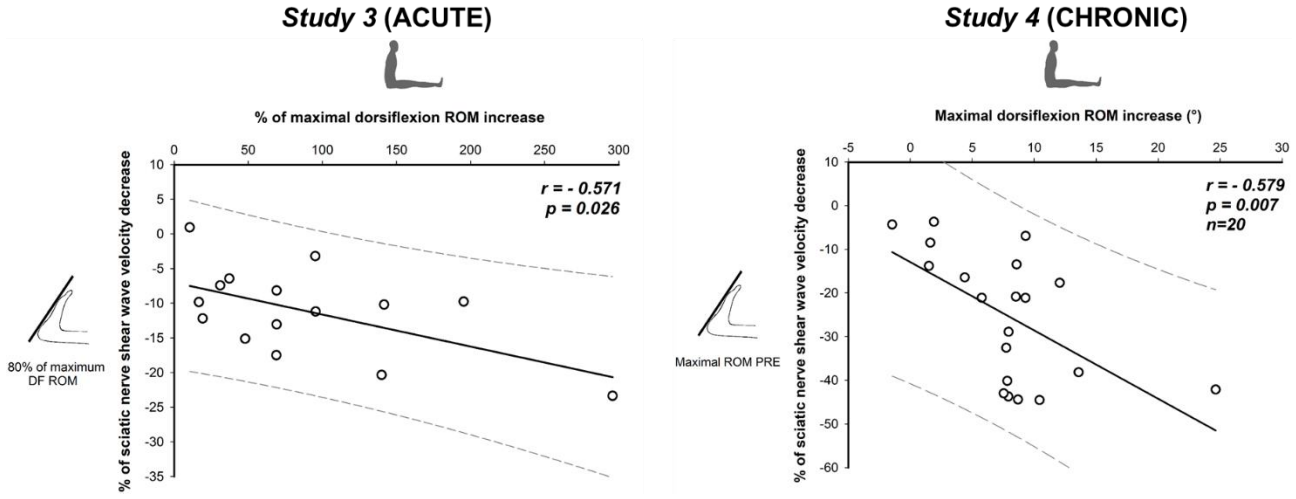



*Figure 30. The effects of both ACUTE and CHRONIC nerve stretching technique on local sciatic nerve stiffness and maximal ankle ROM in dorsiflexion.* ***Study 3 (ACUTE).*** *(n=15)The negative correlation coefficient shows that the acute nerve stretching induced a percentage increase in the maximal ankle ROM in dorsiflexion as measured in the HIP-flexed position that is correlated with the percentage decrease in the sciatic nerve (stretch session).* ***Study 4 (CHRONIC).*** *The negative correlation coefficient shows that the chronic stretching of the sciatic nerve induced an increase in maximal ankle ROM in dorsiflexion (°) measured in the HIP-flexed position that is correlated with a percentage decrease in sciatic nerve stiffness.*

However, it should be noted that stiffness assessments were only performed in the HIP-neutral position, while the changes in maximal ROM occurred in both the HIP-neutral and HIP-flexed positions following sciatic stretching. Thus, our results show evidence underlying the sciatic nerve's role in ankle function.

## 3.2. Maximal ROM adaptations muscle and nerve stretching

In respect to maximal ROM, the findings of the present thesis were threefold: 1) in some multi-joint configurations of the lower limb, the maximal ROM at a given joint can be limited by non-muscular structures; 2) specific stretch training to either muscular or non-muscular (sciatic nerve) structures induces individual mechanical changes in the target tissue; and maximal ROM adaptations are dependent on the mechanical adaptations in the tissue that is more stressed (muscular or non-muscular) during ROM assessment.

It is a long-held belief that when there's no bony apposition, the maximal ROM is limited by the tension within the muscles being stretched (Gajdosik, 2001). As such, the maximal ankle ROM in dorsiflexion might be limited by the maximal tension that can be reached in the plantar flexors. However, in regard to maximal dorsiflexion ROM, the findings of this thesis show that it can be notably affected by the hip joint when the knee remains fully extended. In studies 1, 3, and 4, the maximal ROM in dorsiflexion was assessed in both HIP-flexed and HIP-neutral positions (Figure 25). In these three studies, we observed a remarkable decrease in maximal ROM when the hip was flexed from neutral to a more flexed position (>50%). In study 1, the hip range change between the testing positions was slightly inferior (HIP-neutral = 150°; HIP-flexed = 90°) to those used in studies 3 and 4 (HIP-neutral = 180°; HIP-flexed = 90°). Interestingly, the average decrease in ankle ROM induced by the hip joint flexion was slightly greater in studies 3 and 4 (>60%) than what was observed in study 1 (~50%). Overall, though, these results are in agreement with previous findings reported by Mitchell et al. (2008), who observed a ~22° deficit when maximal ROM was evaluated in the HIP-flexed compared to a more hip extended position (HIP-neutral). Interestingly, by changing the movement sequence, Gajdosik et al. (1985) and Boyd et al. (2009) found identical maximal joint ROM impairment at the hip level. Briefly, they observed a ~10° significant decrease in maximal hip flexion ROM when the ankle was previously positioned in a dorsiflexed position compared to testing with the ankle positioned in plantar flexion (knee was kept in full extension).

However, those studies only examined the effect of hip angle on maximal ankle ROM and vice versa. In contrast, the originality of study 1 was to assess both muscle and joint stiffness during maximal ROM assessment in both hip testing positions. The purpose was to understand whether joint and muscle mechanical properties could be factors underpinning such maximal ROM differences. Remarkably, it was observed that the decrease in maximal ROM due to the hip flexion was not linked to changes in local gastrocnemius stiffness and ankle passive torque. Specifically, these observations led to the conclusion that in certain joint configurations, such as the long-sitting position (HIP-flexion), other non-muscular and multi-joint structures should be firstly loaded maximally during passive ankle rotation towards dorsiflexion. This would have notably affected the extensibility of mono- or bi-articular plantar flexors acting on the tested ankle joint. The early stop in maximal ROM assessment when the hip is flexed should have been triggered by the tension within the non-muscular structures being stretched. For example, both peripheral nerves and fasciae are richly innervated with receptors that, when strained, might trigger the central nervous system to limit ROM (Yahia et al., 1992; Dilley et al., 2005; Stecco et al., 2007; Bove, 2008; Bove & Dilley, 2010).

Notably, this thesis further shows that maximal ROM in dorsiflexion can be increased by stretching non-muscular structures. Interestingly, we observed that these improvements in ankle flexibility can be induced by both acute and chronic stretch training. Surprisingly, in both stretching regimens the increase in maximal ankle ROM in dorsiflexion occurred not only in the testing position where non-muscular structures should be maximally stretched (HIP-flexed), but also in the hip testing position in which maximal ROM should be limited by the tension of the muscle structures (HIP-neutral). However, the effects of non-muscular stretching on maximal ROM in dorsiflexion were greater in the HIP-flexed position (+6.4° ± 2.4° and +8.0° ± 5.2° following acute and chronic stretching, respectively) than in the HIP-neutral position (+5.3° ± 4.2° and +3.8° ± 4.9° following acute and chronic stretching, respectively). Moreover, all the subjects exposed to acute stretch protocol (study 3) improved their maximal ROM in the HIP-flexed position. In respect to study 4, chronic stretch training targeting non-muscular structures increased maximal dorsiflexion ROM in the HIP-flexed position for all subjects, with the exception of one. This exception can, however, be explained by the initial flexibility, since this was the most flexible subject in the HIP-flexed testing position prior to intervention. Taken together, these results suggest that although stretching induces an increase in maximal ROM, a considerable inter-subject

variability in respect to the magnitude of such ROM improvements was observed. This magnitude effect of non-muscular stretching in the increase of ROM trends to be higher in subjects with lower levels of maximal ROM assessed in the HIP-flexed position.

The novelty of studies 3 and 4 resided not only in examining the effects of non-muscular stretching in maximal ankle ROM, but also in quantifying the stiffness adaptations of nerve tissue after acute and chronic interventions. The decrease in sciatic nerve stiffness following both acute (-13.3 % ± 7.9 %) and chronic (-19.1 % ± 15.3 %) stretch interventions contributes notably to expanding the knowledge underpinning the mechanical mechanisms limiting the maximal ROM in dorsiflexion when assessed in the HIP-flexed position. These results strongly suggest that the non-muscular stretch interventions (Figure 27) significantly tensioned the sciatic nerve system, and that the intensity was sufficient to trigger mechanical adaptations within the neural tissue in both cases. Therefore, they validate, for the first time *in-vivo*, that the use of neurodynamic tensioning maneuvers or orthopedics testing procedures, such as straight leg raising, can mechanically load the sciatic nerve and affect maximal ROM. Moreover, in studies 3 and 4, the nerve stiffness adaptations occurred in the absence of mechanical changes in *triceps surae* muscles. This strongly suggests that muscle tissues were not involved in maximal ROM limitation when it is assessed in the HIP-flexed position. Moreover, both studies are in agreement with a recent systematic review that a significant treatment effect of using neurodynamic techniques including nerve stretching/tensioning improves flexibility (Neto et al., 2017). Additionally, it proves that mechanical testing of the sciatic nerve can be performed without affecting other musculoskeletal structures, confirming *in-vivo* a previous cadaver observation (Coppieters et al., 2006) and clinical belief (Butler, 1991; Shacklock, 2005). Although there is sufficient cadaver-based evidence to admit that the non-muscular stretching position loaded the neural tissue (sciatic nerve system) (Breig & Troup, 1979; Coppieters et al., 2006; Alshami et al., 2008; Boyd et al., 2013), potential mechanical adaptations in other non-muscular tissues such as the myofascial layers cannot be totally excluded as primary factors limiting maximal ROM in the HIP-flexed position testing.

Although it has a small effect, it was interesting to note that the non-muscular stretch regimens of studies 3 and 4 also induced a significant increase in maximal ROM as assessed in the HIP-neutral position. Interestingly, study 2 revealed that the sciatic nerve loads mechanically at a

proximal site when the ankle is rotated towards maximal dorsiflexion in the HIP-neutral position and with the knee fully extended. The steep increase in nerve stiffness occurs from ~50% of the ankle's maximum dorsiflexion ROM. As such, it is possible that the decrease in sciatic nerve stiffness following both acute and chronic non-muscular interventions may also have played a role in the maximal ROM assessed in the HIP-neutral position. Remarkably, these results contrast with a long-held belief that maximal ROM in dorsiflexion is only restricted by plantar flexors muscles when assessed in the HIP-neutral position.

In accordance with classical findings (Young et al., 2013; Medeiros et al., 2016; Freitas et al., 2017), study 4 also showed that chronic stretching designed to target muscular structures (i.e., muscle STR) significantly improved the maximal ROM (+9.4° ± 3.8° following the 12 weeks of stretch training). However, such improvements were only observed in ROM assessment in the HIP-neutral position. Such increase in maximal ROM can be partially explained by the significant decrease in plantar flexors stiffness and the reduction in passive ankle torque that were observed following the training. Thus, these results contrast with those suggested by the findings of Freitas et al. (2017) and Weppler and Magnusson (2010). Our results demonstrate that adaptations in maximal joint ROM in response to chronic stretching preferentially evoke mechanical adaptations at both the tissue and joint levels, rather than alterations in sensory adaptations, as previously described. Longer duration protocol and a higher stretching dose may have contributed to our findings.

However, the contribution of possible changes in *triceps surae* architecture, such as increased fascicle length following a chronic stretch training, as suggested by Freitas and Mil-Homens (2015), could have played an important role in both stiffness adaptions and muscle extensibility. These data are currently being processed and may further elucidate the mechanical mechanisms underlying improvements in maximal joint ROM (e.g., source of decrease in muscle stiffness) following chronic stretching interventions targeting muscle tissues. Interestingly, no changes occurred in ankle flexibility assessed in the HIP-flexed position (-2.1° ± 3.8°). Taken together, these observations further validate the important role of non-muscular structures as primary factors limiting maximal ROM in HIP-flexed assessments (suggested by study 1).

Overall, the present thesis certainly supports the biomechanical complexity of assessing the maximal ROM variable. The maximal ROM can be limited by either muscular or non-muscular

structures. Specifically, we demonstrated that the multi-joint configurations of the entire limb will determine the magnitude of stretching that will be imposed on both muscles and non-muscular structures and, thus, the maximal ROM at a given joint. Although our findings strongly suggest that the hip joint will determine whether ankle dorsiflexion will stretch more plantar flexors muscles or non-muscular structures such as the sciatic nerve, they cannot totally dissociate muscle from non-muscular stretching.

## 4. Tissue and joint mechanical adaptations to stretching

This doctoral research has also sought to examine the effects of stretching in mechanical adaptations of two tissues: muscle and nerve. Such adaptations were notably induced by specific stretching preferentially targeting each tissue (e.g., muscle STR and sciatic STR in study 4). Chronic changes were evaluated in nerve and muscle tissue stretching (study 4). Additionally, acute effects of stretching in nerve tissue were assessed in study 3.

The results from the studies mentioned above provide more support for the mechanical based-theory rather than the sensory-based theory to explain increased maximal ROM, contrasting with the findings of a recent systematic review and meta-analysis (Freitas et al., 2017). In addition, Nordez et al. (2017) suggested that non-muscular structures can limit maximal ROM. Additionally, Shah (2017) recently suggested that nerves may have the potential to adapt mechanically following persistent stretching stimuli. The findings of this thesis expand the arguments of these current opinions, providing more direct evidence about the mechanical role of non-muscular structures in maximal ROM. Specific acute and chronic stretch training can trigger stiffness adaptations in local nerve tissue that can partially explain the increases in joint flexibility.

### 4.1. Chronic adaptations of skeletal muscles

As was shown in this thesis, the mechanical properties of skeletal muscle can adapt to chronic stretch stimuli. Notably, the findings from study 4 demonstrate that muscle stiffness decreased following chronic stretching. Interestingly, ankle passive torque also decreased, reflecting the stiffness changes of all MTU acting across the joint (Magnusson, 1998; Gajdosik, 2001).

Therefore, volume (>12 weeks) and intensity (onset on stretch pain) combined sufficiently to trigger adaptation within the muscle–tendon unit. In contrast, it has been argued that chronic stretching for 3 to 8 weeks does not modify significantly the MTU mechanical properties, although it does significantly increase the MTU extensibility and tolerance to a greater tensile force (Weppler & Magnusson, 2010; Freitas et al., 2017). However, most of the underlying assumptions regarding an increase in muscle extensibility have been based on global torque-angle assessments. It is possible that decreases in local muscle stiffness following stretch training would have not been sufficient to induce significant changes at a joint level (i.e., joint passive torque).

Using ultrasound shear wave elastography, two recent studies observed a significant decrease in local muscle stiffness following chronic stretching for 5–6 weeks (Akagi & Takahashi, 2014; Ichihashi et al., 2016). The results of study 4 are in agreement with such findings. Taken together, these studies suggest that the response of muscle tissue to chronic stretching is not homogenous within across a muscle group. Although Akagi Akagi and Takahashi (2014) examined adaptations in gastrocnemii transverse stiffness (described as muscle hardness) before the slack length and Ichihashi *et al.* (2016) explored stiffness changes across hamstring, another novel and important finding of this research is that stiffness adaptations are not homogeneous within the same muscle. Such inter- and intra-muscular heterogeneity of muscle stiffness adaptations strengthens the interest of using localized shear wave velocity (directly linked to the shear modulus) measurements above classic passive torque measurements in order to analyze the chronic effects of stretching. The mechanisms underpinning the decrease in muscle stiffness may have origin at two levels: mechanical and physiological (Gajdosik, 2001; Weppler & Magnusson, 2010). The mechanical dimension refers to adaptations in muscle slack length and changes in intrinsic muscle elasticity, while physiological level concerns adaptations in connective tissues and fiber length. However, data from slack length and muscle architecture are still being processed. Thus, to date, the mechanical findings of study 4 do not allow for a determination on whether the decrease in muscle stiffness is related to an increased muscle fiber length or a shift to the right in the passive stiffness–joint angle relationship.

## 4.2. Acute and chronic adaptations of the nerve

The non-muscular loading techniques performed in studies 3 and 4 aiming to stretch the sciatic nerve system demonstrated that nerve tissue adapts to both acute and chronic stimuli, respectively. The findings from this thesis suggest that nerves respond to stretching with a decrease in their stiffness. This is in line with previous research conducted in animals and *in-vitro*, showing that nerves exhibit non-linear stress–strain behavior and time-dependent viscoelastic properties when stretched to a fixed strain (Rydevik et al., 1990; Wall et al., 1991; Kwan et al., 1992; Millesi et al., 1995; Driscoll et al., 2002; Ma et al., 2013).

On a different level, the chronic mechanical adaptations in the peripheral nerve could have been related to structural changes in the nerve core and/or sheaths (Tillett et al., 2004; Georgeu et al., 2005), notably in the dense connective tissue network present along the nerve length. Indeed, most of the mechanical protection of peripheral nerves is provided by the epineurium, perineurium, and endoneurium. While it has been suggested that wavy collagen arrangement (Type I and Type III) and elastic fibers that comprise the epineurium provide mechanical protection from compression (Stolinski, 1995; Topp & Boyd, 2006), the perineurium has been considered to be the primary contributor to the nerve's viscoelastic properties (Rydevik et al., 1990; Sunderland, 1990; Topp & Boyd, 2006). Moreover, structural changes could have occurred in longitudinal undulations (i.e., bands of Fontana) in the core and nerve fascicles, leading to an increase in extensibility and a decrease in nerve stiffness (Clarke & Bearn, 1972; Haninec, 1986; Zachary et al., 1993; Shah, 2017).

Additionally, our findings from study 4 demonstrate a non-homogeneous stiffness response to sciatic nerve system stretching. This finding confirms a previous animal observation showing that strain on nerves during limb movement is not equally distributed along their length, but is increased close to joints (Phillips et al., 2004). This may be related to a localized variation in collagen fibril diameter that has been suggested to contribute to the longitudinal heterogeneity of tensile properties in this nerve (Mason & Phillips, 2011). In contrast, the nerve stiffness adaptations to chronic stretching were homogeneous along the nerve.

Additionally, dynamic stretching should also play a role on nerve stiffness. Data from study 2 suggests a tendency for the nerve stiffness to decrease throughout the repetition of five

loading/unloading cycles of stretching. However, the stretching stimulus used in study 2 should not have been sufficient to trigger stiffness changes of loading cycles in the sciatic nerve, as previously demonstrated by Orf and Wust (1979). Further studies are required to determine whether dynamic stretch training can affect mechanical properties of nerves and joint function.

## 4.3. Contribution of muscular and non-muscular adaptations to joint torque

Assessment of passive torque-angle relationships is traditionally used to assess the mechanical properties of MTU (Magnusson, 1998; Gajdosik, 2001), and is commonly used to identify pre- to post-stretching adaptations in such structures (Weppler & Magnusson, 2010; Freitas et al., 2017). However, the results of the present thesis (studies 1, 3, and 4) demonstrate that stretching techniques used to preferentially load muscular or non-muscular structures induce different responses at the joint level.

Notably, the analysis of nerve stiffness and torque data from studies 3 and 4 demonstrated that stretching non-muscular tissues has a trivial impact on global passive joint torque. Specifically, non-muscular stretching interventions did not induce significant changes in passive torque, but they did alter nerve tissue stiffness. These results are in accordance with the current theory suggested by (Nordez *et al.*, 2017), but differs from others that have evaluated the effect of adding sensitizing maneuvers, which are classically used in some clinical tests such as straight leg raising or slump to stretch the sciatic nerve, on joint torque (Laessoe & Voigt, 2004; McHugh et al., 2012; Andrade et al., 2015; Palmer et al., 2015). Although the latter studies have observed an increase in joint torque, the maneuvers used to sensitize the sciatic nerve should have directly increased the length and tensioning of muscles acting in the joint where torque was evaluated, and thus explain such results.

Interestingly, we observed that ankle torque adaptations to specific chronic muscle stretching reflects the stiffness changes of MTU acting across the target joint. However, passive torque should not be representative of the entire adaptations that were seen heterogeneously at the muscle level (i.e., intra-muscular and inter-muscular).

This strengthens the cause to use localized shear wave velocity (as an index of stiffness) measurements rather than classic passive torque measurements for analyzing the chronic effects of stretching in individual tissues, both muscular and non-muscular.

## 5. Limitations & perspectives

Some general limitations were identified in this research. The main limitation of is thesis is that the sciatic nerve stiffness was not assessed in the HIP-flexed position, where the nerve tract should stretch maximally as the ankle joint is passively dorsiflexed towards maximal ROM. Thus, the hypothesis raised in study 1 that nerve stiffness can affect the maximal joint ROM was not directly addressed. However, the findings of studies 3 and 4 (e.g., correlations between nerve stiffness and maximal dorsiflexion ROM as illustrated in Figure 29 and Figure 30) contribute to conclusions about the important role of the sciatic nerve in the limitation of maximal dorsiflexion ROM by using a more indirect methodological approach.

Additionally, peripheral nerves such as the sciatic tract display three-dimensional movement when stretched at relatively large length ranges (Boyd et al., 2012). As such, they should exhibit not only longitudinal motion, but also move in a transverse plane before accommodating large levels of tensile force. Thus, because we used 2-D elastography imaging, the positioning of the probe remains operator-dependent, which can be problematic to track shear waves in relatively thin structures. The measurement of shear wave propagation in the entire volume using 3-D or 4-D ultrafast shear-wave imaging may overcome these limitations (Deffieux et al., 2008; Gennisson et al., 2013; Gennisson et al., 2015) and should be examined in future studies.

Moreover, this doctoral research is limited to the findings of sciatic nerve stiffness and ankle dorsiflexion ROM. Although we are content that our observations are likely to occur in other nerves and joints, further studies are necessary to confirm such assumptions.

In addition, both nerve and muscle stiffness are organized in series with other structures, such as nerve roots and tendons, respectively. Although the stiffness of the latter structures could have been affected by the stretching protocols of studies 3 and 4, the local stiffness that was assessed in both nerve and muscles tissues should also reflect the changes in tensile force for elements organized in series.

One major limitation is that the findings of this thesis do not allow for concluding whether maximal ROM is regulated mechanically by one or several muscular and non-muscular structures. For instance, due to their anatomical organization and continuity through the lower limb (Stecco & Hammer, 2015), both the deep fascia of the thigh (fascia lata) and the deep fascia of the leg (crural fascia) could have been stretch during the interventions performed in both study 2 and study 3. Thus, further research is needed to clarify the role of fasciae and other non-muscular structures (e.g., skin, vascular structures) in joint function.

Although our findings were strongly supported by mechanical adaptations in the stretched structures, sensory central mechanisms acting specifically in the stretching perception of the lengthened tissues cannot be totally excluded in changes in maximal dorsiflexion ROM. For instance, further studies should examine whether stretching protocols induce adaptations in stretch pain perception for the same tensile force in the stretched tissue.

Another issue is the level of muscle activation during maximal ROM assessments. Although involuntary electrical activity was negligible (<3%) and a lack of differences was observed between muscular and non-muscular assessments, the magnitude at which muscle activation influences passive mechanical measurements remains largely unknown. Thus, while this should not be a major problem for the assessments performed in healthy populations, recent findings of our research team suggest that it is likely to have an effect on neurological populations, such as in stroke survivors (Le Sant et al., submitted for publication). In order to examine the influence of non-muscular structures in clinical populations, further studies are required to better isolate passive from active forces during passive stretching. For instance, transient blocking of motor nerves could be used to exclude involuntary muscle activity during stretching, thus providing important information for assessing tissue mechanical properties in isolated passive conditions.

## 6. Conclusion

Using ultrasound shear wave elastography to assess local stiffness in muscle and nerves, this doctoral research showed that maximal joint ROM can be limited by either muscular or non-muscular structures, which strongly depended on the posture of the entire lower limb. Additionally, acute and chronic stretching targeting either muscles or nerves were shown to induce individual

changes in their mechanical properties. Moreover, improvements in maximal joint ROM following stretching interventions are specific to the tissues that were more tensioned, and are correlated with the changes in their mechanical properties.

Thus, this thesis extends the knowledge about the mechanical role of muscular and non-muscular tissues underpinning the limitation of maximal joint ROM *in-vivo*. Consequently, it provides a significant contribution towards enhancing future design and prescription of individualized stretching programs, and allows for more individual interpretations of the clinical value of loading either muscular or non-muscular structures in maximal joint ROM assessments.

*Chapter Six*

# REFERENCES

Abbott BC & Lowy J. (1956). Stress relaxation in muscle. *Proceedings of the Royal Society of London Series B, Biological sciences* **146,** 281-288.

Abe I, Ochiai N, Ichimura H, Tsujino A, Sun J & Hara Y. (2004). Internodes can nearly double in length with gradual elongation of the adult rat sciatic nerve. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **22,** 571-577.

Akagi R & Takahashi H. (2014). Effect of a 5-week static stretching program on hardness of the gastrocnemius muscle. *Scandinavian journal of medicine & science in sports* **24,** 950-957.

Akeson WH, Amiel D, Abel MF, Garfin SR & Woo SL. (1987). Effects of immobilization on joints. *Clinical orthopaedics and related research***,** 28-37.

Alshami AM, Babri AS, Souvlis T & Coppieters MW. (2008). Strain in the tibial and plantar nerves with foot and ankle movements and the influence of adjacent joint positions. *J Appl Biomech* **24,** 368-376.

Andrade RJ, Freitas SR, Vaz JR, Bruno PM & Pezarat-Correia P. (2015). Provocative mechanical tests of the peripheral nervous system affect the joint torque-angle during passive knee motion. *Scandinavian journal of medicine & science in sports* **25,** 338-345.

Andrade RJ, Lacourpaille L, Freitas SR, McNair PJ & Nordez A. (2016a). Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension. *Scandinavian journal of medicine & science in sports* **26,** 41-47.

Andrade RJ, Nordez A, Hug F, Ates F, Coppieters MW, Pezarat-Correia P & Freitas SR. (2016b). Non-invasive assessment of sciatic nerve stiffness during human ankle motion using ultrasound shear wave elastography. *Journal of biomechanics* **49,** 326-331.

Aumailley M & Gayraud B. (1998). Structure and biological activity of the extracellular matrix. *Journal of molecular medicine* **76,** 253-265.

Bamber J, Cosgrove D, Dietrich CF, Fromageau J, Bojunga J, Calliada F, Cantisani V, Correas JM, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Gilja OH, Havre RF, Jenssen C, Klauser AS, Ohlinger R, Saftoiu A, Schaefer F, Sporea I & Piscaglia F. (2013). EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. *Ultraschall in der Medizin* **34,** 169-184.

Beel JA, Stodieck LS & Luttges MW. (1986). Structural properties of spinal nerve roots: biomechanics. *Experimental neurology* **91,** 30-40.

Behm DG, Blazevich AJ, Kay AD & McHugh M. (2016a). Acute effects of muscle stretching on physical performance, range of motion, and injury incidence in healthy active individuals: a systematic review. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **41,** 1-11.

Behm DG, Cavanaugh T, Quigley P, Reid JC, Nardi PS & Marchetti PH. (2016b). Acute bouts of upper and lower body static and dynamic stretching increase non-local joint range of motion. *European journal of applied physiology* **116,** 241-249.

Ben M & Harvey LA. (2010). Regular stretch does not increase muscle extensibility: a randomized controlled trial. *Scandinavian journal of medicine & science in sports* **20,** 136-144.

Benetazzo L, Bizzego A, De Caro R, Frigo G, Guidolin D & Stecco C. (2011). 3D reconstruction of the crural and thoracolumbar fasciae. *Surgical and radiologic anatomy : SRA* **33,** 855-862.

Bensamoun SF, Wang L, Robert L, Charleux F, Latrive JP & Ho Ba Tho MC. (2008). Measurement of liver stiffness with two imaging techniques: magnetic resonance elastography and ultrasound elastometry. *Journal of magnetic resonance imaging : JMRI* **28,** 1287-1292.

Bercoff J, Tanter M & Fink M. (2004). Supersonic shear imaging: a new technique for soft tissue elasticity mapping. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **51,** 396-409.

Blazevich AJ, Cannavan D, Waugh CM, Miller SC, Thorlund JB, Aagaard P & Kay AD. (2014). Range of motion, neuromechanical, and architectural adaptations to plantar flexor stretch training in humans. *Journal of applied physiology* **117,** 452-462.

Bojsen-Moller J, Schwartz S, Kalliokoski KK, Finni T & Magnusson SP. (2010). Intermuscular force transmission between human plantarflexor muscles in vivo. *Journal of applied physiology* **109,** 1608-1618.

Bouvier T, Opplert J, Cometti C & Babault N. (2017). Acute effects of static stretching on muscle-tendon mechanics of quadriceps and plantar flexor muscles. *European journal of applied physiology* **117,** 1309-1315.

Bove GM. (2008). Epi-perineurial anatomy, innervation, and axonal nociceptive mechanisms. *Journal of bodywork and movement therapies* **12,** 185-190.

Bove GM & Dilley A. (2010). The conundrum of sensitization when recording from nociceptors. *Journal of neuroscience methods* **188,** 213-218.

Bove GM & Light AR. (1995a). Calcitonin gene-related peptide and peripherin immunoreactivity in nerve sheaths. *Somatosensory & motor research* **12,** 49-57.

Bove GM & Light AR. (1995b). Unmyelinated nociceptors of rat paraspinal tissues. *Journal of neurophysiology* **73,** 1752-1762.

Bove GM & Light AR. (1997). The nervi nervorum: Missing link for neuropathic pain? *Pain Forum* **6,** 181-190.

Boyce D & Brosky JA, Jr. (2008). Determining the minimal number of cyclic passive stretch repetitions recommended for an acute increase in an indirect measure of hamstring length. *Physiotherapy theory and practice* **24,** 113-120.

Boyd BS, Gray AT, Dilley A, Wanek L & Topp KS. (2012). The pattern of tibial nerve excursion with active ankle dorsiflexion is different in older people with diabetes mellitus. *Clinical biomechanics* **27,** 967-971.

Boyd BS, Topp KS & Coppieters MW. (2013). Impact of movement sequencing on sciatic and tibial nerve strain and excursion during the straight leg raise test in embalmed cadavers. *The Journal of orthopaedic and sports physical therapy* **43,** 398-403.

Boyd BS, Wanek L, Gray AT & Topp KS. (2009). Mechanosensitivity of the lower extremity nervous system during straight-leg raise neurodynamic testing in healthy individuals. *The Journal of orthopaedic and sports physical therapy* **39,** 780-790.

Brandenburg JE, Eby SF, Song P, Zhao H, Brault JS, Chen S & An KN. (2014). Ultrasound elastography: the new frontier in direct measurement of muscle stiffness. *Archives of physical medicine and rehabilitation* **95,** 2207-2219.

Breig A & Braxton V. (1978). *Adverse Mechanical Tension in the Central Nervous System: An Analysis of Cause and Effect : Relief by Functional Neurosurgery*. Almqvist & Wiksell International.

Breig A & Troup JD. (1979). Biomechanical considerations in the straight-leg-raising test. Cadaveric and clinical studies of the effects of medial hip rotation. *Spine* **4,** 242-250.

Bressel E & McNair PJ. (2002). The effect of prolonged static and cyclic stretching on ankle joint stiffness, torque relaxation, and gait in people with stroke. *Physical therapy* **82,** 880-887.

Brum J, Bernal M, Gennisson JL & Tanter M. (2014). In vivo evaluation of the elastic anisotropy of the human Achilles tendon using shear wave dispersion analysis. *Physics in medicine and biology* **59,** 505-523.

Bueno FR & Shah SB. (2008). Implications of tensile loading for the tissue engineering of nerves. *Tissue engineering Part B, Reviews* **14,** 219-233.

Butler DS. (1991). *Mobilisation of the Nervous System*. Churchill Livingstone.

Ce E, Longo S, Rampichini S, Devoto M, Limonta E, Venturelli M & Esposito F. (2015). Stretch-induced changes in tension generation process and stiffness are not accompanied by alterations in muscle architecture of the middle and distal portions of the two gastrocnemii. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology* **25,** 469-478.

Chan SP, Hong Y & Robinson PD. (2001). Flexibility and passive resistance of the hamstrings of young adults using two different static stretching protocols. *Scandinavian journal of medicine & science in sports* **11,** 81-86.

Chaouachi A, Padulo J, Kasmi S, Othmen AB, Chatra M & Behm DG. (2017). Unilateral static and dynamic hamstrings stretching increases contralateral hip flexion range of motion. *Clinical physiology and functional imaging* **37,** 23-29.

Cho Sims G, Boothe E, Joodi R & Chhabra A. (2016). 3D MR Neurography of the Lumbosacral Plexus: Obtaining Optimal Images for Selective Longitudinal Nerve Depiction. *AJNR American journal of neuroradiology*.

Clarke E & Bearn JG. (1972). The spiral nerve bands of Fontana. *Brain : a journal of neurology* **95,** 1-20.

Coppieters MW, Alshami AM, Babri AS, Souvlis T, Kippers V & Hodges PW. (2006). Strain and excursion of the sciatic, tibial, and plantar nerves during a modified straight leg raising test. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **24,** 1883-1889.

Coppieters MW, Andersen LS, Johansen R, Giskegjerde PK, Hoivik M, Vestre S & Nee RJ. (2015). Excursion of the Sciatic Nerve During Nerve Mobilization Exercises: An In Vivo Cross-sectional Study Using Dynamic Ultrasound Imaging. *The Journal of orthopaedic and sports physical therapy* **45,** 731-737.

Coppieters MW & Butler DS. (2008). Do 'sliders' slide and 'tensioners' tension? An analysis of neurodynamic techniques and considerations regarding their application. *Manual therapy* **13,** 213-221.

Cruz-Montecinos C, Gonzalez Blanche A, Lopez Sanchez D, Cerda M, Sanzana-Cuche R & Cuesta-Vargas A. (2015). In vivo relationship between pelvis motion and deep fascia displacement of the medial gastrocnemius: anatomical and functional implications. *Journal of anatomy* **227,** 665-672.

Davis J, Kaufman KR & Lieber RL. (2003). Correlation between active and passive isometric force and intramuscular pressure in the isolated rabbit tibialis anterior muscle. *Journal of biomechanics* **36,** 505-512.

Decoster LC, Cleland J, Altieri C & Russell P. (2005). The effects of hamstring stretching on range of motion: a systematic literature review. *The Journal of orthopaedic and sports physical therapy* **35,** 377-387.

Deffieux T, Gennisson JL, Tanter M & Fink M. (2008). Assessment of the mechanical properties of the musculoskeletal system using 2-D and 3-D very high frame rate ultrasound. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **55,** 2177-2190.

DeWall RJ, Slane LC, Lee KS & Thelen DG. (2014). Spatial variations in Achilles tendon shear wave speed. *Journal of biomechanics* **47,** 2685-2692.

Dikici AS, Ustabasioglu FE, Delil S, Nalbantoglu M, Korkmaz B, Bakan S, Kula O, Uzun N, Mihmanli I & Kantarci F. (2016). Evaluation of the Tibial Nerve with Shear-Wave Elastography: A Potential Sonographic Method for the Diagnosis of Diabetic Peripheral Neuropathy. *Radiology***,** 160135.

Dilley A, Lynn B & Pang SJ. (2005). Pressure and stretch mechanosensitivity of peripheral nerve fibres following local inflammation of the nerve trunk. *Pain* **117,** 462-472.

Driscoll PJ, Glasby MA & Lawson GM. (2002). An in vivo study of peripheral nerves in continuity: biomechanical and physiological responses to elongation. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **20,** 370-375.

Dubois G, Kheireddine W, Vergari C, Bonneau D, Thoreux P, Rouch P, Tanter M, Gennisson JL & Skalli W. (2015). Reliable protocol for shear wave elastography of lower limb muscles at rest and during passive stretching. *Ultrasound in medicine & biology* **41,** 2284-2291.

e Lima KM, Carneiro SP, Alves Dde S, Peixinho CC & de Oliveira LF. (2015). Assessment of muscle architecture of the *biceps femoris* and vastus lateralis by ultrasound after a chronic stretching program. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine* **25,** 55-60.

Eby SF, Song P, Chen S, Chen Q, Greenleaf JF & An KN. (2013). Validation of shear wave elastography in skeletal muscle. *Journal of biomechanics* **46,** 2381-2387.

Ellis RF, Hing WA & McNair PJ. (2012). Comparison of longitudinal sciatic nerve movement with different mobilization exercises: an in vivo study utilizing ultrasound imaging. *The Journal of orthopaedic and sports physical therapy* **42,** 667-675.

Enoka RM. (2015). *Neuromechanics of Human Movement 5th Edition*. Human Kinetics.

Fergusson D, Hutton B & Drodge A. (2007). The epidemiology of major joint contractures: a systematic review of the literature. *Clinical orthopaedics and related research* **456,** 22-29.

Finni T. (2006). Structural and functional features of human muscle-tendon unit. *Scandinavian journal of medicine & science in sports* **16,** 147-158.

Freitas SR, Andrade RJ, Larcoupaille L, Mil-homens P & Nordez A. (2015a). Muscle and joint responses during and after static stretching performed at different intensities. *European journal of applied physiology* **115,** 1263-1272.

Freitas SR, Andrade RJ, Nordez A, Mendes B & Mil-Homens P. (2016). Acute muscle and joint mechanical responses following a high-intensity stretching protocol. *European journal of applied physiology* **116,** 1519-1526.

Freitas SR, Mendes B, Le Sant G, Andrade RJ, Nordez A & Milanovic Z. (2017). Can chronic stretching change the muscle-tendon mechanical properties? A review. *Scandinavian journal of medicine & science in sports*.

Freitas SR & Mil-Homens P. (2015). Effect of 8-week high-intensity stretching training on *biceps femoris* architecture. *Journal of strength and conditioning research* **29,** 1737-1740.

Freitas SR, Vaz JR, Bruno PM, Valamatos MJ, Andrade RJ & Mil-Homens P. (2015b). Are rest intervals between stretching repetitions effective to acutely increase range of motion? *International journal of sports physiology and performance* **10,** 191-197.

Freitas SR, Vaz JR, Gomes L, Silvestre R, Hilario E, Cordeiro N, Carnide F, Pezarat-Correia P & Mil-Homens P. (2015c). A New Tool to Assess the Perception of Stretching Intensity. *Journal of strength and conditioning research* **29,** 2666-2678.

Freitas SR, Vilarinho D, Rocha Vaz J, Bruno PM, Costa PB & Mil-homens P. (2015d). Responses to static stretching are dependent on stretch intensity and duration. *Clinical physiology and functional imaging* **35,** 478-484.

Gajdosik RL. (2001). Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clinical biomechanics* **16,** 87-101.

Gajdosik RL, Allred JD, Gabbert HL & Sonsteng BA. (2007). A stretching program increases the dynamic passive length and passive resistive properties of the calf muscle-tendon unit of unconditioned younger women. *European journal of applied physiology* **99,** 449-454.

Gajdosik RL, LeVeau BF & Bohannon RW. (1985). Effects of ankle dorsiflexion on active and passive unilateral straight leg raising. *Physical therapy* **65,** 1478-1482.

Gajdosik RL, Vander Linden DW, McNair PJ, Riggin TJ, Albertson JS, Mattick DJ & Wegley JC. (2004). Slow passive stretch and release characteristics of the calf muscles of older women with limited dorsiflexion range of motion. *Clinical biomechanics* **19,** 398-406.

Gajdosik RL, Vander Linden DW, McNair PJ, Williams AK & Riggin TJ. (2005). Effects of an eight-week stretching program on the passive-elastic properties and function of the calf muscles of older women. *Clinical biomechanics* **20,** 973-983.

Gamble HJ & Eames RA. (1964). An Electron Microscope Study of the Connective Tissues of Human Peripheral Nerve. *Journal of anatomy* **98,** 655-663.

Gao F, Ren Y, Roth EJ, Harvey R & Zhang LQ. (2011). Effects of repeated ankle stretching on calf muscle-tendon and ankle biomechanical properties in stroke survivors. *Clinical biomechanics* **26,** 516-522.

Garrett WE, Jr., Rich FR, Nikolaou PK & Vogler JB, 3rd. (1989). Computed tomography of hamstring muscle strains. *Medicine and science in sports and exercise* **21,** 506-514.

Gennisson JL, Catheline S, Chaffai S & Fink M. (2003). Transient elastography in anisotropic medium: application to the measurement of slow and fast shear wave speeds in muscles. *The Journal of the Acoustical Society of America* **114,** 536-541.

Gennisson JL, Deffieux T, Fink M & Tanter M. (2013). Ultrasound elastography: principles and techniques. *Diagnostic and interventional imaging* **94,** 487-495.

Gennisson JL, Deffieux T, Mace E, Montaldo G, Fink M & Tanter M. (2010). Viscoelastic and anisotropic mechanical properties of in vivo muscle tissue assessed by supersonic shear imaging. *Ultrasound in medicine & biology* **36,** 789-801.

Gennisson JL, Provost J, Deffieux T, Papadacci C, Imbault M, Pernot M & Tanter M. (2015). 4-D ultrafast shear-wave imaging. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **62,** 1059-1065.

Georgeu GA, Walbeehm ET, Tillett R, Afoke A, Brown RA & Phillips JB. (2005). Investigating the mechanical shear-plane between core and sheath elements of peripheral nerves. *Cell and tissue research* **320,** 229-234.

Gerlach UJ & Lierse W. (1990). Functional construction of the superficial and deep fascia system of the lower limb in man. *Acta anatomica* **139,** 11-25.

Gilbert KK, Brismee JM, Collins DL, James CR, Shah RV, Sawyer SF & Sizer PS, Jr. (2007). 2006 Young Investigator Award Winner: lumbosacral nerve root displacement and strain: part 1. A novel measurement technique during straight leg raise in unembalmed cadavers. *Spine* **32,** 1513-1520.

Gillies AR & Lieber RL. (2011). Structure and function of the skeletal muscle extracellular matrix. *Muscle & nerve* **44,** 318-331.

Goddard MD & Reid JD. (1965). Movements Induced by Straight Leg Raising in the Lumbo-Sacral Roots, Nerves and Plexus, and in the Intrapelvic Section of the Sciatic Nerve. *Journal of neurology, neurosurgery, and psychiatry* **28,** 12-18.

Goldspink G, Tabary C, Tabary JC, Tardieu C & Tardieu G. (1974). Effect of denervation on the adaptation of sarcomere number and muscle extensibility to the functional length of the muscle. *The Journal of physiology* **236,** 733-742.

Gordon AM, Huxley AF & Julian FJ. (1966). Tension development in highly stretched vertebrate muscle fibres. *The Journal of physiology* **184,** 143-169.

Granzier H, Kellermayer M, Helmes M & Trombitas K. (1997). Titin elasticity and mechanism of passive force development in rat cardiac myocytes probed by thin-filament extraction. *Biophysical journal* **73,** 2043-2053.

Granzier HL & Irving TC. (1995). Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. *Biophysical journal* **68,** 1027-1044.

Granzier HL & Pollack GH. (1985). Stepwise shortening in unstimulated frog skeletal muscle fibres. *The Journal of physiology* **362,** 173-188.

Gray H, Carter HV, Pick TP & Howden R. (2012). *Gray's Anatomy*. Octopus Publishing Group.

Greening J & Dilley A. (2017). Posture-induced changes in peripheral nerve stiffness measured by ultrasound shear-wave elastography. *Muscle & nerve* **55,** 213-222.

Guissard N & Duchateau J. (2004). Effect of static stretch training on neural and mechanical properties of the human plantar-flexor muscles. *Muscle & nerve* **29,** 248-255.

Haftek J. (1970). Stretch injury of peripheral nerve. Acute effects of stretching on rabbit nerve. *The Journal of bone and joint surgery British volume* **52,** 354-365.

Halbertsma JP & Goeken LN. (1994). Stretching exercises: effect on passive extensibility and stiffness in short hamstrings of healthy subjects. *Archives of physical medicine and rehabilitation* **75,** 976-981.

Halbertsma JP, van Bolhuis AI & Goeken LN. (1996). Sport stretching: effect on passive muscle stiffness of short hamstrings. *Archives of physical medicine and rehabilitation* **77,** 688-692.

Haninec P. (1986). Undulating course of nerve fibres and bands of Fontana in peripheral nerves of the rat. *Anatomy and embryology* **174,** 407-411.

Hara Y, Shiga T, Abe I, Tsujino A, Ichimura H, Okado N & Ochiai N. (2003). P0 mRNA expression increases during gradual nerve elongation in adult rats. *Experimental neurology* **184,** 428-435.

Harvey LA, Batty J, Crosbie J, Poulter S & Herbert RD. (2000). A randomized trial assessing the effects of 4 weeks of daily stretching on ankle mobility in patients with spinal cord injuries. *Archives of physical medicine and rehabilitation* **81,** 1340-1347.

Harvey LA & Herbert RD. (2002). Muscle stretching for treatment and prevention of contracture in people with spinal cord injury. *Spinal cord* **40,** 1-9.

Helfenstein-Didier C, Andrade RJ, Brum J, Hug F, Tanter M, Nordez A & Gennisson JL. (2016). In vivo quantification of the shear modulus of the human Achilles tendon during passive loading using shear wave dispersion analysis. *Physics in medicine and biology* **61,** 2485-2496.

Hemmerich A, Brown H, Smith S, Marthandam SS & Wyss UP. (2006). Hip, knee, and ankle kinematics of high range of motion activities of daily living. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **24,** 770-781.

Herbert R. (1988). The passive mechanical properties of muscle and their adaptations to altered patterns of use. *The Australian journal of physiotherapy* **34,** 141-149.

Herbert RD, Clarke J, Kwah LK, Diong J, Martin J, Clarke EC, Bilston LE & Gandevia SC. (2011). In vivo passive mechanical behaviour of muscle fascicles and tendons in human gastrocnemius muscle-tendon units. *The Journal of physiology* **589,** 5257-5267.

Herbert RD & Crosbie J. (1997). Rest length and compliance of non-immobilised and immobilised rabbit soleus muscle and tendon. *European journal of applied physiology and occupational physiology* **76,** 472-479.

Herbert RD, Heroux ME, Diong J, Bilston LE, Gandevia SC & Lichtwark GA. (2015). Changes in the length and three-dimensional orientation of muscle fascicles and aponeuroses with passive length changes in human gastrocnemius muscles. *The Journal of physiology* **593,** 441-455.

Herbert RD, Moseley AM, Butler JE & Gandevia SC. (2002). Change in length of relaxed muscle fascicles and tendons with knee and ankle movement in humans. *The Journal of physiology* **539,** 637-645.

Herda TJ, Herda ND, Costa PB, Walter-Herda AA, Valdez AM & Cramer JT. (2013). The effects of dynamic stretching on the passive properties of the muscle-tendon unit. *Journal of sports sciences* **31,** 479-487.

Hermens HJ, Freriks B, Disselhorst-Klug C & Rau G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology* **10,** 361-374.

Herzog JA, Leonard TR, Jinha A & Herzog W. (2012). Are titin properties reflected in single myofibrils? *Journal of biomechanics* **45,** 1893-1899.

Hirata K, Kanehisa H, Miyamoto-Mikami E & Miyamoto N. (2015). Evidence for intermuscle difference in slack angle in human *triceps surae*. *Journal of biomechanics* **48,** 1210-1213.

Hirata K, Kanehisa H & Miyamoto N. (2017). Acute effect of static stretching on passive stiffness of the human gastrocnemius fascicle measured by ultrasound shear wave elastography. *European journal of applied physiology* **117,** 493-499.

Hirata K, Miyamoto-Mikami E, Kanehisa H & Miyamoto N. (2016). Muscle-specific acute changes in passive stiffness of human *triceps surae* after stretching. *European journal of applied physiology* **116,** 911-918.

Hoang PD, Gorman RB, Todd G, Gandevia SC & Herbert RD. (2005). A new method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. *Journal of biomechanics* **38,** 1333-1341.

Hoang PD, Herbert RD, Todd G, Gorman RB & Gandevia SC. (2007). Passive mechanical properties of human gastrocnemius muscle tendon units, muscle fascicles and tendons in vivo. *The Journal of experimental biology* **210,** 4159-4168.

Horowits R & Podolsky RJ. (1987). The positional stability of thick filaments in activated skeletal muscle depends on sarcomere length: evidence for the role of titin filaments. *The Journal of cell biology* **105,** 2217-2223.

Hromada J. (1963). On the Nerve Supply of the Connective Tissue of Some Peripheral Nervous System Components. *Acta anatomica* **55,** 343-351.

Hug F, Lacourpaille L, Maisetti O & Nordez A. (2013). Slack length of *gastrocnemius medialis* and Achilles tendon occurs at different ankle angles. *Journal of biomechanics* **46,** 2534-2538.

Hug F, Tucker K, Gennisson JL, Tanter M & Nordez A. (2015). Elastography for Muscle Biomechanics: Toward the Estimation of Individual Muscle Force. *Exercise and sport sciences reviews* **43,** 125-133.

Huijing PA, Yaman A, Ozturk C & Yucesoy CA. (2011). Effects of knee joint angle on global and local strains within human *triceps surae* muscle: MRI analysis indicating in vivo myofascial force transmission between synergistic muscles. *Surgical and radiologic anatomy : SRA* **33,** 869-879.

Hurschler C, Vanderby R, Jr., Martinez DA, Vailas AC & Turnipseed WD. (1994). Mechanical and biochemical analyses of tibial compartment fascia in chronic compartment syndrome. *Annals of biomedical engineering* **22,** 272-279.

Ichihashi N, Umegaki H, Ikezoe T, Nakamura M, Nishishita S, Fujita K, Umehara J, Nakao S & Ibuki S. (2016). The effects of a 4-week static stretching programme on the individual muscles comprising the hamstrings. *Journal of sports sciences* **34,** 2155-2159.

Johns RJ & Wright V. (1962). Relative importance of various tissues in joint stiffness. *Journal of applied physiology* **17,** 824-828.

Kantarci F, Ustabasioglu FE, Delil S, Olgun DC, Korkmazer B, Dikici AS, Tutar O, Nalbantoglu M, Uzun N & Mihmanli I. (2014). Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. *Eur Radiol* **24,** 434-440.

Kara M, Ozcakar L, Tiftik T, Kaymak B, Ozel S, Akkus S & Akinci A. (2012). Sonographic evaluation of sciatic nerves in patients with unilateral sciatica. *Archives of physical medicine and rehabilitation* **93,** 1598-1602.

Kassab GS & Sacks MS. (2016). *Structure-Based Mechanics of Tissues and Organs*. Springer US.

Katalinic OM, Harvey LA, Herbert RD, Moseley AM, Lannin NA & Schurr K. (2010). Stretch for the treatment and prevention of contractures. *The Cochrane database of systematic reviews,* CD007455.

Kay AD & Blazevich AJ. (2009). Moderate-duration static stretch reduces active and passive plantar flexor moment but not Achilles tendon stiffness or active muscle length. *Journal of applied physiology* **106,** 1249-1256.

Kendall JP, Stokes IA, O'Hara JP & Dickson RA. (1979). Tension and creep phenomena in peripheral nerve. *Acta orthopaedica Scandinavica* **50,** 721-725.

Kjaer M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological reviews* **84,** 649-698.

Klauser AS, Miyamoto H, Bellmann-Weiler R, Feuchtner GM, Wick MC & Jaschke WR. (2014). Sonoelastography: musculoskeletal applications. *Radiology* **272,** 622-633.

Kobayashi S, Shizu N, Suzuki Y, Asai T & Yoshizawa H. (2003). Changes in nerve root motion and intraradicular blood flow during an intraoperative straight-leg-raising test. *Spine* **28,** 1427-1434.

Konrad A, Gad M & Tilp M. (2015). Effect of PNF stretching training on the properties of human muscle and tendon structures. *Scandinavian journal of medicine & science in sports* **25,** 346-355.

Konrad A & Tilp M. (2014a). Effects of ballistic stretching training on the properties of human muscle and tendon structures. *Journal of applied physiology* **117,** 29-35.

Konrad A & Tilp M. (2014b). Increased range of motion after static stretching is not due to changes in muscle and tendon structures. *Clinical biomechanics* **29,** 636-642.

Koo TK, Guo JY, Cohen JH & Parker KJ. (2013). Relationship between shear elastic modulus and passive muscle force: an ex-vivo study. *Journal of biomechanics* **46,** 2053-2059.

Koo TK, Guo JY, Cohen JH & Parker KJ. (2014). Quantifying the passive stretching response of human tibialis anterior muscle using shear wave elastography. *Clinical biomechanics* **29,** 33-39.

Koo TK & Hug F. (2015). Factors that influence muscle shear modulus during passive stretch. *Journal of biomechanics* **48,** 3539-3542.

Kot BC, Zhang ZJ, Lee AW, Leung VY & Fu SN. (2012). Elastic modulus of muscle and tendon with shear wave ultrasound elastography: variations with different technical settings. *PloS one* **7,** e44348.

Kubo K, Kanehisa H & Fukunaga T. (2002). Effect of stretching training on the viscoelastic properties of human tendon structures in vivo. *Journal of applied physiology* **92,** 595-601.

Kubo K, Kanehisa H & Fukunaga T. (2005). Effects of cold and hot water immersion on the mechanical properties of human muscle and tendon in vivo. *Clinical biomechanics* **20,** 291-300.

Kubo K, Kanehisa H, Kawakami Y & Fukunaga T. (2001). Influence of static stretching on viscoelastic properties of human tendon structures in vivo. *Journal of applied physiology* **90,** 520-527.

Kwan MK, Wall EJ, Massie J & Garfin SR. (1992). Strain, stress and stretch of peripheral nerve. Rabbit experiments in vitro and in vivo. *Acta orthopaedica Scandinavica* **63,** 267-272.

Lacourpaille L, Hug F, Bouillard K, Hogrel JY & Nordez A. (2012). Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. *Physiol Meas* **33,** N19-28.

Lacourpaille L, Nordez A, Hug F, Doguet V, Andrade R & Guilhem G. (2017). Early detection of exercise-induced muscle damage using elastography. *European journal of applied physiology*.

Laessoe U & Voigt M. (2004). Modification of stretch tolerance in a stooping position. *Scandinavian journal of medicine & science in sports* **14,** 239-244.

LaRoche DP & Connolly DA. (2006). Effects of stretching on passive muscle tension and response to eccentric exercise. *The American journal of sports medicine* **34,** 1000-1007.

Law RY, Harvey LA, Nicholas MK, Tonkin L, De Sousa M & Finniss DG. (2009). Stretch exercises increase tolerance to stretch in patients with chronic musculoskeletal pain: a randomized controlled trial. *Physical therapy* **89,** 1016-1026.

Le Sant G, Ates F, Brasseur JL & Nordez A. (2015). Elastography Study of Hamstring Behaviors during Passive Stretching. *PloS one* **10,** e0139272.

Le Sant G, Nordez A, Andrade R, Hug F, Freitas S & Gross R. (2017). Stiffness mapping of lower leg muscles during passive dorsiflexion. *Journal of anatomy* **230,** 639-650.

Lieber RL & Bodine-Fowler SC. (1993). Skeletal muscle mechanics: implications for rehabilitation. *Physical therapy* **73,** 844-856.

Lima K, Costa Junior JFS, Pereira WCA & Oliveira LF. (2017). Assessment of the mechanical properties of the muscle-tendon unit by supersonic shear wave imaging elastography: a review. *Ultrasonography*.

Ma Z, Hu S, Tan JS, Myer C, Njus NM & Xia Z. (2013). In vitro and in vivo mechanical properties of human ulnar and median nerves. *J Biomed Mater Res A* **101,** 2718-2725.

Maas H & Sandercock TG. (2010). Force transmission between synergistic skeletal muscles through connective tissue linkages. *Journal of biomedicine & biotechnology* **2010,** 575672.

Magid A & Law DJ. (1985). Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* **230,** 1280-1282.

Magnusson SP. (1998). Passive properties of human skeletal muscle during stretch maneuvers. A review. *Scandinavian journal of medicine & science in sports* **8,** 65-77.

Magnusson SP, Aagard P, Simonsen E & Bojsen-Moller F. (1998). A biomechanical evaluation of cyclic and static stretch in human skeletal muscle. *International journal of sports medicine* **19,** 310-316.

Magnusson SP, Simonsen EB, Aagaard P, Gleim GW, McHugh MP & Kjaer M. (1995). Viscoelastic response to repeated static stretching in the human hamstring muscle. *Scandinavian journal of medicine & science in sports* **5,** 342-347.

Magnusson SP, Simonsen EB, Aagaard P & Kjaer M. (1996a). Biomechanical responses to repeated stretches in human hamstring muscle in vivo. *The American journal of sports medicine* **24,** 622-628.

Magnusson SP, Simonsen EB, Aagaard P, Sorensen H & Kjaer M. (1996b). A mechanism for altered flexibility in human skeletal muscle. *The Journal of physiology* **497 ( Pt 1),** 291-298.

Magnusson SP, Simonsen EB, Dyhre-Poulsen P, Aagaard P, Mohr T & Kjaer M. (1996c). Viscoelastic stress relaxation during static stretch in human skeletal muscle in the absence of EMG activity. *Scandinavian journal of medicine & science in sports* **6,** 323-328.

Mahieu NN, Cools A, De Wilde B, Boon M & Witvrouw E. (2009). Effect of proprioceptive neuromuscular facilitation stretching on the plantar flexor muscle-tendon tissue properties. *Scandinavian journal of medicine & science in sports* **19,** 553-560.

Mahieu NN, McNair P, De Muynck M, Stevens V, Blanckaert I, Smits N & Witvrouw E. (2007). Effect of static and ballistic stretching on the muscle-tendon tissue properties. *Medicine and science in sports and exercise* **39,** 494-501.

Maikos JT, Elias RA & Shreiber DI. (2008). Mechanical properties of dura mater from the rat brain and spinal cord. *Journal of neurotrauma* **25,** 38-51.

Maisetti O, Hug F, Bouillard K & Nordez A. (2012). Characterization of passive elastic properties of the human medial gastrocnemius muscle belly using supersonic shear imaging. *Journal of biomechanics* **45,** 978-984.

Marshall PW, Cashman A & Cheema BS. (2011). A randomized controlled trial for the effect of passive stretching on measures of hamstring extensibility, passive stiffness, strength, and stretch tolerance. *Journal of science and medicine in sport* **14,** 535-540.

Mason S & Phillips JB. (2011). An ultrastructural and biochemical analysis of collagen in rat peripheral nerves: the relationship between fibril diameter and mechanical properties. *Journal of the peripheral nervous system : JPNS* **16,** 261-269.

Matsuo S, Suzuki S, Iwata M, Banno Y, Asai Y, Tsuchida W & Inoue T. (2013). Acute effects of different stretching durations on passive torque, mobility, and isometric muscle force. *Journal of strength and conditioning research* **27,** 3367-3376.

Mazgajczyk E, Scigala K, Czyz M, Jarmundowicz W & Bedzinski R. (2012). Mechanical properties of cervical dura mater. *Acta of bioengineering and biomechanics* **14,** 51-58.

McHugh MP & Cosgrave CH. (2010). To stretch or not to stretch: the role of stretching in injury prevention and performance. *Scandinavian journal of medicine & science in sports* **20,** 169-181.

McHugh MP, Johnson CD & Morrison RH. (2012). The role of neural tension in hamstring flexibility. *Scandinavian journal of medicine & science in sports* **22,** 164-169.

McHugh MP, Kremenic IJ, Fox MB & Gleim GW. (1998). The role of mechanical and neural restraints to joint range of motion during passive stretch. *Medicine and science in sports and exercise* **30,** 928-932.

McNair PJ, Dombroski EW, Hewson DJ & Stanley SN. (2001). Stretching at the ankle joint: viscoelastic responses to holds and continuous passive motion. *Medicine and science in sports and exercise* **33,** 354-358.

Medeiros DM, Cini A, Sbruzzi G & Lima CS. (2016). Influence of static stretching on hamstring flexibility in healthy young adults: Systematic review and meta-analysis. *Physiotherapy theory and practice* **32,** 438-445.

Mescher A. (2013). *Junqueira's Basic Histology: Text and Atlas, Thirteenth Edition: Text and Atlas, Thirteenth Edition*. McGraw-Hill Education.

Millesi H, Zoch G & Reihsner R. (1995). Mechanical properties of peripheral nerves. *Clinical orthopaedics and related research***,** 76-83.

Mitchell B, Bressel E, McNair PJ & Bressel ME. (2008). Effect of pelvic, hip, and knee position on ankle joint range of motion. *Phys Ther Sport* **9,** 202-208.

Mizuno T, Matsumoto M & Umemura Y. (2013a). Decrements in stiffness are restored within 10 min. *International journal of sports medicine* **34,** 484-490.

Mizuno T, Matsumoto M & Umemura Y. (2013b). Viscoelasticity of the muscle-tendon unit is returned more rapidly than range of motion after stretching. *Scandinavian journal of medicine & science in sports* **23,** 23-30.

Morse CI, Degens H, Seynnes OR, Maganaris CN & Jones DA. (2008). The acute effect of stretching on the passive stiffness of the human gastrocnemius muscle tendon unit. *The Journal of physiology* **586,** 97-106.

Moseley GL. (2008). Pain, brain imaging and physiotherapy--opportunity is knocking. *Manual therapy* **13,** 475-477.

Muanjai P, Jones DA, Mickevicius M, Satkunskiene D, Snieckus A, Rutkauskaite R, Mickeviciene D & Kamandulis S. (2017). The effects of 4 weeks stretching training to the point of pain on flexibility and muscle tendon unit properties. *European journal of applied physiology* **117,** 1713-1725.

Muir IW, Chesworth BM & Vandervoort AA. (1999). Effect of a static calf-stretching exercise on the resistive torque during passive ankle dorsiflexion in healthy subjects. *The Journal of orthopaedic and sports physical therapy* **29,** 106-113; discussion 114-105.

Mulholland SJ & Wyss UP. (2001). Activities of daily living in non-Western cultures: range of motion requirements for hip and knee joint implants. *International journal of rehabilitation research Internationale Zeitschrift fur Rehabilitationsforschung Revue internationale de recherches de readaptation* **24,** 191-198.

Mutungi G & Ranatunga KW. (1996). The viscous, viscoelastic and elastic characteristics of resting fast and slow mammalian (rat) muscle fibres. *The Journal of physiology* **496 ( Pt 3),** 827-836.

Mutungi G & Ranatunga KW. (1998). Temperature-dependent changes in the viscoelasticity of intact resting mammalian (rat) fast- and slow-twitch muscle fibres. *The Journal of physiology* **508 ( Pt 1),** 253-265.

Nakamura M, Ikezoe T, Takeno Y & Ichihashi N. (2011). Acute and prolonged effect of static stretching on the passive stiffness of the human gastrocnemius muscle tendon unit in vivo. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **29,** 1759-1763.

Nakamura M, Ikezoe T, Takeno Y & Ichihashi N. (2012). Effects of a 4-week static stretch training program on passive stiffness of human gastrocnemius muscle-tendon unit in vivo. *European journal of applied physiology* **112,** 2749-2755.

Nakamura M, Ikezoe T, Tokugawa T & Ichihashi N. (2015). Acute Effects of Stretching on Passive Properties of Human Gastrocnemius Muscle-Tendon Unit: Analysis of Differences Between Hold-Relax and Static Stretching. *Journal of sport rehabilitation* **24,** 286-292.

Nakamura M, Ikezoe T, Umegaki H, Kobayashi T, Nishishita S & Ichihashi N. (2016). Changes in Passive Properties of the Gastrocnemius Muscle-Tendon Unit During a 4-Week Routine Static Stretching Program. *Journal of sport rehabilitation***,** 1-17.

Neto T, Freitas SR, Marques M, Gomes L, Andrade R & Oliveira R. (2017). Effects of lower body quadrant neural mobilization in healthy and low back pain populations: A systematic review and meta-analysis. *Musculoskeletal Science and Practice* **27,** 14-22.

Netter FH. (2010). *Atlas of Human Anatomy*. Elsevier Health Sciences.

Nikolaou PK, Macdonald BL, Glisson RR, Seaber AV & Garrett WE, Jr. (1987). Biomechanical and histological evaluation of muscle after controlled strain injury. *The American journal of sports medicine* **15,** 9-14.

Noonan TJ, Best TM, Seaber AV & Garrett WE, Jr. (1994). Identification of a threshold for skeletal muscle injury. *The American journal of sports medicine* **22,** 257-261.

Nordez A, Casari P & Cornu C. (2008a). Effects of stretching velocity on passive resistance developed by the knee musculo-articular complex: contributions of frictional and viscoelastic behaviours. *European journal of applied physiology* **103,** 243-250.

Nordez A, Foure A, Dombroski EW, Mariot JP, Cornu C & McNair PJ. (2010a). Improvements to Hoang et al.'s method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. *Journal of biomechanics* **43,** 379-382.

Nordez A, Gross R, Andrade RJ, Le Sant G, Freitas SR, Ellis R, McNair PJ & Hug F. (2017). Non-Muscular Structures Can Limit the Maximal Joint Range of Motion during Stretching. *Sports medicine* **[Epub ahead of print]**.

Nordez A, McNair P, Casari P & Cornu C. (2008b). Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. *International journal of sports medicine* **29,** 414-418.

Nordez A, McNair PJ, Casari P & Cornu C. (2009). The effect of angular velocity and cycle on the dissipative properties of the knee during passive cyclic stretching: a matter of viscosity or solid friction. *Clinical biomechanics* **24,** 77-81.

Nordez A, McNair PJ, Casari P & Cornu C. (2010b). Static and cyclic stretching: their different effects on the passive torque-angle curve. *Journal of science and medicine in sport* **13,** 156-160.

Norkin CC & White DJ. (2009). *Measurement of Joint Motion: A Guide to Goniometry*. F.A. Davis.

Nubar Y. (1962). Stress-strain relationship in skeletal muscle. *Annals of the New York Academy of Sciences* **93,** 859-876.

Ophir J, Cespedes I, Ponnekanti H, Yazdi Y & Li X. (1991). Elastography: a quantitative method for imaging the elasticity of biological tissues. *Ultrasonic imaging* **13,** 111-134.

Opplert J, Genty JB & Babault N. (2016). Do Stretch Durations Affect Muscle Mechanical and Neurophysiological Properties? *International journal of sports medicine* **37,** 673-679.

Orf G & Wust R. (1979). Mechanical loading of peripheral nerves during remobilisation of the affected member after end-to-end anastomosis. *Acta neurochirurgica* **49,** 103-121.

Page P. (2012). Current concepts in muscle stretching for exercise and rehabilitation. *International journal of sports physical therapy* **7,** 109-119.

Palmer TB, Akehi K, Thiele RM, Smith DB, Warren AJ & Thompson BJ. (2015). Dorsiflexion, plantar-flexion, and neutral ankle positions during passive resistance assessments of the posterior hip and thigh muscles. *Journal of athletic training* **50,** 467-474.

Phillips JB, Smit X, De Zoysa N, Afoke A & Brown RA. (2004). Peripheral nerves in the rat exhibit localized heterogeneity of tensile properties during limb movement. *The Journal of physiology* **557,** 879-887.

Porter SB. (2013). *Tidy's Physiotherapy15: Tidy's Physiotherapy*. Elsevier.

Portero P & McNair PJ. (2010). Utilisation des dynamomètres isocinétiques pour la caractérisation des propriétés mécaniques passives du complexe muscle-tendon. *La Lettre de médecine physique et de réadaptation* **26,** 5-8.

Proske U & Morgan DL. (1999). Do cross-bridges contribute to the tension during stretch of passive muscle? *Journal of muscle research and cell motility* **20,** 433-442.

Purslow PP. (1989). Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *Journal of biomechanics* **22,** 21-31.

Purslow PP. (2002). The structure and functional significance of variations in the connective tissue within muscle. *Comparative biochemistry and physiology Part A, Molecular & integrative physiology* **133,** 947-966.

Purslow PP. (2010). Muscle fascia and force transmission. *Journal of bodywork and movement therapies* **14,** 411-417.

Purslow PP, Wess TJ & Hukins DW. (1998). Collagen orientation and molecular spacing during creep and stress-relaxation in soft connective tissues. *The Journal of experimental biology* **201,** 135-142.

Rade M, Kononen M, Vanninen R, Marttila J, Shacklock M, Kankaanpaa M & Airaksinen O. (2014). 2014 young investigator award winner: In vivo magnetic resonance imaging measurement of spinal cord displacement in the thoracolumbar region of asymptomatic subjects: part 1: straight leg raise test. *Spine* **39,** 1288-1293.

Radford JA, Burns J, Buchbinder R, Landorf KB & Cook C. (2006). Does stretching increase ankle dorsiflexion range of motion? A systematic review. *British journal of sports medicine* **40,** 870-875; discussion 875.

Reid DA & McNair PJ. (2004). Passive force, angle, and stiffness changes after stretching of hamstring muscles. *Medicine and science in sports and exercise* **36,** 1944-1948.

Reid DA & McNair PJ. (2010). Effects of an acute hamstring stretch in people with and without osteoarthritis of the knee. *Physiotherapy* **96,** 14-21.

Ridehalgh C, Moore A & Hough A. (2014). Normative sciatic nerve excursion during a modified straight leg raise test. *Manual therapy* **19,** 59-64.

Riemann BL, DeMont RG, Ryu K & Lephart SM. (2001). The Effects of Sex, Joint Angle, and the Gastrocnemius Muscle on Passive Ankle Joint Complex Stiffness. *Journal of athletic training* **36,** 369-375.

Ryan ED, Beck TW, Herda TJ, Hull HR, Hartman MJ, Costa PB, Defreitas JM, Stout JR & Cramer JT. (2008). The time course of musculotendinous stiffness responses following different durations of passive stretching. *The Journal of orthopaedic and sports physical therapy* **38,** 632-639.

Ryan ED, Herda TJ, Costa PB, Walter AA, Hoge KM, Stout JR & Cramer JT. (2010). Viscoelastic creep in the human skeletal muscle-tendon unit. *European journal of applied physiology* **108,** 207-211.

Rydevik BL, Kwan MK, Myers RR, Brown RA, Triggs KJ, Woo SL & Garfin SR. (1990). An in vitro mechanical and histological study of acute stretching on rabbit tibial nerve. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **8,** 694-701.

Saleh HA, El-fark MM & Abdel-Hamid GA. (2009). Anatomical variation of sciatic nerve division in the popliteal fossa and its implication in popliteal nerve blockade. *Folia morphologica* **68,** 256-259.

Shacklock M. (2005). *Clinical Neurodynamics: A New System of Musculoskeletal Treatment*. Elsevier Butterworth-Heinemann.

Shah SB. (2017). Tissue Biomechanics: Whales Have Some Nerve. *Curr Biol* **27,** R177-R179.

Shibukawa M & Shirai Y. (2001). Experimental study on slow-speed elongation injury of the peripheral nerve: electrophysiological and histological changes. *Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association* **6,** 262-268.

Sigrist RMS, Liau J, Kaffas AE, Chammas MC & Willmann JK. (2017). Ultrasound Elastography: Review of Techniques and Clinical Applications. *Theranostics* **7,** 1303-1329.

Silva A, Manso A, Andrade R, Domingues V, Brandao MP & Silva AG. (2014). Quantitative in vivo longitudinal nerve excursion and strain in response to joint movement: A systematic literature review. *Clinical biomechanics* **29,** 839-847.

Smith SA, Massie JB, Chesnut R & Garfin SR. (1993). Straight leg raising. Anatomical effects on the spinal nerve root without and with fusion. *Spine* **18,** 992-999.

Stecco C, Gagey O, Belloni A, Pozzuoli A, Porzionato A, Macchi V, Aldegheri R, De Caro R & Delmas V. (2007). Anatomy of the deep fascia of the upper limb. Second part: study of innervation. *Morphologie : bulletin de l'Association des anatomistes* **91,** 38-43.

Stecco C & Hammer WI. (2015). *Functional Atlas of the Human Fascial System*. Elsevier - Health Sciences Division.

Stecco C, Macchi V, Porzionato A, Duparc F & De Caro R. (2011). The fascia: the forgotten structure. *Italian journal of anatomy and embryology = Archivio italiano di anatomia ed embriologia* **116,** 127-138.

Stecco C, Pavan P, Pachera P, De Caro R & Natali A. (2014). Investigation of the mechanical properties of the human crural fascia and their possible clinical implications. *Surgical and radiologic anatomy : SRA* **36,** 25-32.

Stodieck LS, Beel JA & Luttges MW. (1986). Structural properties of spinal nerve roots: protein composition. *Experimental neurology* **91,** 41-51.

Stolinski C. (1995). Structure and composition of the outer connective tissue sheaths of peripheral nerve. *Journal of anatomy* **186 ( Pt 1),** 123-130.

Sun JS, Tsuang YH, Liu TK, Hang YS, Cheng CK & Lee WW. (1995). Viscoplasticity of rabbit skeletal muscle under dynamic cyclic loading. *Clinical biomechanics* **10,** 258-262.

Sunderland S. (1990). The anatomy and physiology of nerve injury. *Muscle & nerve* **13,** 771-784.

Tabary JC, Tabary C, Tardieu C, Tardieu G & Goldspink G. (1972). Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *The Journal of physiology* **224,** 231-244.

Tanaka S & Ito T. (1977). Histochemical demonstration of adrenergic fibers in the fascia periosteum and retinaculum. *Clinical orthopaedics and related research,* 276-281.

Taniguchi K, Shinohara M, Nozaki S & Katayose M. (2015). Acute decrease in the stiffness of resting muscle belly due to static stretching. *Scandinavian journal of medicine & science in sports* **25,** 32-40.

Taylor DC, Dalton JD, Jr., Seaber AV & Garrett WE, Jr. (1990). Viscoelastic properties of muscle-tendon units. The biomechanical effects of stretching. *The American journal of sports medicine* **18,** 300-309.

Tesarz J, Hoheisel U, Wiedenhofer B & Mense S. (2011). Sensory innervation of the thoracolumbar fascia in rats and humans. *Neuroscience* **194,** 302-308.

Thomas PK. (1963). The connective tissue of peripheral nerve: an electron microscope study. *Journal of anatomy* **97,** 35-44.

Thorpe CT & Screen HR. (2016). Tendon Structure and Composition. *Advances in experimental medicine and biology* **920,** 3-10.

Tian M, Hoang PD, Gandevia SC, Bilston LE & Herbert RD. (2010). Stress relaxation of human ankles is only minimally affected by knee and ankle angle. *Journal of biomechanics* **43,** 990-993.

Tillett RL, Afoke A, Hall SM, Brown RA & Phillips JB. (2004). Investigating mechanical behaviour at a core-sheath interface in peripheral nerve. *Journal of the peripheral nervous system : JPNS* **9,** 255-262.

Topp KS & Boyd BS. (2006). Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Physical therapy* **86,** 92-109.

Trestik CL & Lieber RL. (1993). Relationship between Achilles tendon mechanical properties and gastrocnemius muscle function. *Journal of biomechanical engineering* **115,** 225-230.

Troup JD. (1981). Straight-leg-raising (SLR) and the qualifying tests for increased root tension: their predictive value after back and sciatic pain. *Spine* **6,** 526-527.

Trudel G, Uhthoff HK & Brown M. (1999). Extent and direction of joint motion limitation after prolonged immobility: an experimental study in the rat. *Archives of physical medicine and rehabilitation* **80,** 1542-1547.

Wall EJ, Kwan MK, Rydevik BL, Woo SL & Garfin SR. (1991). Stress relaxation of a peripheral nerve. *J Hand Surg Am* **16,** 859-863.

Wang JH. (2006). Mechanobiology of tendon. *Journal of biomechanics* **39,** 1563-1582.

Wang K, McCarter R, Wright J, Beverly J & Ramirez-Mitchell R. (1993). Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophysical journal* **64,** 1161-1177.

Ward SR, Tomiya A, Regev GJ, Thacker BE, Benzl RC, Kim CW & Lieber RL. (2009). Passive mechanical properties of the lumbar multifidus muscle support its role as a stabilizer. *Journal of biomechanics* **42,** 1384-1389.

Weppler CH & Magnusson SP. (2010). Increasing muscle extensibility: a matter of increasing length or modifying sensation? *Physical therapy* **90,** 438-449.

Wilke J, Vogt L, Niederer D & Banzer W. (2017). Is remote stretching based on myofascial chains as effective as local exercise? A randomised-controlled trial. *Journal of sports sciences* **35,** 2021-2027.

Williams PE & Goldspink G. (1976). The effect of denervation and dystrophy on the adaptation of sarcomere number to the functional length of the muscle in young and adult mice. *Journal of anatomy* **122,** 455-465.

Witvrouw E, Danneels L, Asselman P, D'Have T & Cambier D. (2003). Muscle flexibility as a risk factor for developing muscle injuries in male professional soccer players. A prospective study. *The American journal of sports medicine* **31,** 41-46.

Yahia L, Rhalmi S, Newman N & Isler M. (1992). Sensory innervation of human thoracolumbar fascia. An immunohistochemical study. *Acta orthopaedica Scandinavica* **63,** 195-197.

Yaman A, Ozturk C, Huijing PA & Yucesoy CA. (2013). Magnetic resonance imaging assessment of mechanical interactions between human lower leg muscles in vivo. *Journal of biomechanical engineering* **135,** 91003.

Ylinen J, Kankainen T, Kautiainen H, Rezasoltani A, Kuukkanen T & Hakkinen A. (2009). Effect of stretching on hamstring muscle compliance. *Journal of rehabilitation medicine* **41,** 80-84.

Young R, Nix S, Wholohan A, Bradhurst R & Reed L. (2013). Interventions for increasing ankle joint dorsiflexion: a systematic review and meta-analysis. *Journal of foot and ankle research* **6,** 46.

Yucesoy CA. (2010). Epimuscular myofascial force transmission implies novel principles for muscular mechanics. *Exercise and sport sciences reviews* **38,** 128-134.

Zachary LS, Dellon ES, Nicholas EM & Dellon AL. (1993). The structural basis of Felice Fontana's spiral bands and their relationship to nerve injury. *Journal of reconstructive microsurgery* **9,** 131-138.

UNIVERSITE BRETAGNE LOIRE

UNIVERSITÉ DE NANTES

# Thèse de Doctorat

Ricardo J. ANDRADE

# Biomechanical properties of skeletal muscle and peripheral nerve: tissue and joint adaptations to acute and chronic stretch interventions

## Propriétés biomécaniques du muscle squelettique et du nerf périphérique : adaptations du tissu et de l'articulation aux étirements aigus et chroniques

**Abstract**

Maximal joint range of motion (ROM) of a joint, or joint flexibility, is an important functional outcome used in clinical practice, sports and research designs to estimate the maximal length of passive muscle-tendon unit (MTU). Maximal ROM was thought to be mainly restricted by the tension developed by MTUs being stretched during articular motion (mechanical-based theory). However, there is a growing body of experimental research suggesting that stretch training induces increases in maximal ROM in the absence of significant mechanical adaptations in MTU (sensory-based theory). More recently, non-muscular structures such as peripheral nerves have been pointed to limit the stretching amplitude. This doctoral research aimed to extend the knowledge about the mechanical role of muscular and non-muscular tissues underpinning the limitation of maximal ROM in-vivo. Taking advantage of ultrasound shear wave elastography to assess the passive stiffness of individual muscles and nerve, four studies were conducted to: examine whether mechanical loading of muscular and non-muscular structures limit the maximal ROM, and if their mechanical properties adapt to either acute or chronic stretch training. The results show that maximal ROM can be limited by either muscular or non-muscular structures, depending on the posture of the entire lower limb which is decisive to stretch preferentially the target tissue. Acute and chronic stretching targeting separately each structure induces changes in mechanical properties of muscles and nerves. In addition, improvements in maximal ROM were specific to the tissues that were more loaded and correlated with the changes in their mechanical properties.

**Key Words: Range of motion - stiffness - ultrasound shear wave elastography - peripheral nerves - muscle - passive torque - ankle joint**

**Résumé**

L'amplitude articulaire maximale (AAM) ou flexibilité, est un paramètre fonctionnel fréquemment utilisé dans la pratique clinique, sportive et au sein des protocoles de recherche pour estimer la longueur maximale de l'unité muscle-tendon (UMT). Il a longtemps été considéré que l'AAM est limitée par la tension développée par les UMTs étirées pendant le mouvement articulaire (*mechanical theory*). Cependant, un nombre important de recherches expérimentales suggèrent que l'étirement peut induire une augmentation de l'AAM en absence d'adaptations mécaniques significatives dans l'UTM (*sensory-based theory*). Récemment, les structures non-musculaires telles que les nerfs périphériques ont été suggérées comme facteurs limitant l'amplitude d'étirement. Cette thèse vise à mieux comprendre le rôle mécanique des tissus musculaires et non-musculaires qui peuvent influencer la limitation de l'AAM in-vivo. En utilisant la technique d'élastographie permettant d'évaluer la raideur passive des muscles et des nerfs individuellement, quatre études ont été réalisées pour : déterminer si l'étirement des structures musculaires et non musculaires limite l'AAM et si les propriétés mécaniques des tissus étirés s'adaptent à l'étirement aigu ou chronique. Les résultats ont montré que l'AAM peut être limitée par des structures musculaires ou non-musculaires. Le positionnement des articulations du membre inferieur est décisif pour étirer de manière spécifique le tissu ciblé et induire des changements au niveau des propriétés mécaniques des muscles et des nerfs après l'application des protocoles d'étirement aigus et chroniques. De plus l'amélioration de l'AAM était spécifique selon les tissus étirés.

**Mots clés : amplitude articulaire maximale - raideur - élastographie ultrasonore par ondes de cisaillement - nerfs périphériques - muscle - couple passif - cheville**