Universidade de Lisboa

Faculdade de Motricidade Humana

Morphological ultrasound evaluation in acute and chronic muscle overloading

Avaliação ecográfica da morfologia muscular perante situações de sobrecarga agudas e crónicas

Rute Andreia Martins dos Santos

Orientador: Professor Doutor Paulo Alexandre Silva Armada da Silva

Tese elaborada com vista à obtenção do grau de Doutor
em Motricidade Humana na especialidade de Biomecânica

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE/TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

Faculdade de Motricidade Humana – Universidade de Lisboa

Cruz Quebrada, ___ de Outubro de 2017

_____

(Rute Andreia Martins dos Santos)

Dedicated to:

My grandfather and godfather Carlos Martins

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Oral communications:

Santos, R., Armada-da-Silva, P. Reproducibility of ultrasound parameters (echo-intensity and thickness) of quadriceps muscle. European Congress of Radiology, European Society of Radiology, Vienna, Austria, 6th-10th March 6-10, 2015.

Santos, R., Valamatos, M.J., Mil-Homens, P., Armada-da-Silva, P.A.S. Elasticity characterisation of vastus lateralis muscle by ultrasound quasi-static elastography. European Congress of Radiology, European Society of Radiology, Vienna, Austria, March 1-5, 2017.

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Santos, R., Valamatos, M.J., Mil-Homens, P., Armada-da-Silva, P. Elasticity characterisation of vastus lateralis muscle by ultrasound quasi-static elastography. Electronic Presentation Online System EPOS, European Congress of Radiology, European Society of Radiology, Vienna.

Armada-da-Silva, P., Santos, R., Valamatos, M.J., Mil-Homens, P. Increased vastus lateralis' hardness after 15 weeks of resistance training: an ultrasound strain elastography study. 22nd Congress of the European College of Sports Science, Essen, Germany, July 5-8, 2017.

Abstract

Introduction: Ultrasound (US) has an important role in musculoskeletal (MSK) evaluation, allowing the study of muscle morphology and function. Muscle thickness (MT) and muscle echo-intensity (EI) are two important parameters that may quantify muscle structural adaptations to a variety of stimuli. US elastography can also offer semi-quantitative and/or quantitative assessment of tissue stiffness providing relevant information about adaptations of muscle mechanical properties.

Purpose: The general aim of the studies presented in this thesis is to explore the potential of quantitative US imaging for assessing the adaptations and responses of the muscle tissue to increased contractile activity using B-mode US and US elastography. The studies were centred on the quadriceps femoris muscle and addressed the study of the effect of strength training and of acute muscle contractile activity on MT, EI and muscle stiffness.

Materials and methods: Three different studies were conducted and reported along this thesis. A total of 64 young adults of both genders participated in the studies. The first study (N = 20) evaluated the intra- and inter-session (one week apart) reproducibility of MT and EI parameters and the role of plane of view (transverse vs. longitudinal) and ROI dimension on measurements' accuracy using the intraclass correlation coefficient [$ICC_{(3,1)}$], the standard error of measurement (SEM), and the smallest detectable change (SDC). Bland-Altman analysis was used to study the level of agreement between plane views and ROI sizes. The second study (N = 28) investigated the effect of a 15-week strength program on MT and EI in several regions of the heads of the quadriceps femoris. This study included a control group and two training groups performing concentric or eccentric strength training. During this study, changes in vastus lateralis' (VL) stiffness in response to strength training were evaluated using quasi-static elastography (QSE). In the final study (N = 16), acute changes in VL's stiffness associated with passive stretching, performance of short but intense contractile activity, and muscle isometric contractions were investigated by means of supersonic shear wave imaging (SSI).

Results: Moderate to very high reliability was found for MT (intra-session, ICCs: 0.82-0.99; inter-session, ICCs: 0.70-0.98) and EI (intra-session, ICCs: 0.74-0.97; inter-session, ICCs: 0.48-0.94). In general, reliability for MT and EI measures was higher in the transverse plane and when using a larger ROI, respectively. Measurements of EI taken with a small versus a large ROI are associated with a small bias and larger limits of

agreement (LoA). In study 2, 15 weeks of strength training increased MT in the majority but not in all of the scanned regions. Strength training failed in changing EI in most of the quadriceps femoris, excepting in the VI and some regions of the VL. Strength training significantly increased VL's stiffness. No differences were observed in our quantitative US parameters between concentric and eccentric training. The final study demonstrated an acute increase of around 10% in VL's shear modulus as a result of performing maximal isometric, concentric, and eccentric contractions. The shear modulus of the VL also increased when the knee moved from 10º to 50º and then to 90º flexion. Finally, a linear relationship between the shear modulus and the level of isometric muscle contraction was observed.

Conclusions: Ultrasound measures of MT and EI show moderate to very high reliability. The reliability and agreement of MT and EI measurements are improved in transverse scans and with larger ROIs. QSE could demonstrate an increase in muscle stiffness as a result of strength training. SSI proved to be a good method to investigate muscle mechanical properties changes associated with muscle function. These results emphasise the value of an objective and quantifiable muscle US evaluation for studying muscle adaptation to exercise training and muscle function, in general.

Key-words: quantitative ultrasound; ultrasound elastography; strength training, muscle adaptation.

Resumo

Introdução: A ultrassonografia tem um papel importante na avaliação músculo-esquelética, permitindo o estudo da morfologia e função muscular. A espessura muscular e a eco-intensidade muscular são dois parâmetros importantes que podem quantificar as adaptações estruturais musculares, quando o musculo é submetido a determinados estímulos. A elastografia por ultrassonografia pode, também, oferecer uma avaliação semi-quantitativa e/ou quantitativa da rigidez do tecido, fornecendo informações relevantes sobre as adaptações das propriedades mecânicas musculares.

Objetivo: O objetivo geral, dos estudos apresentados nesta tese, é explorar o potencial da imagem quantitativa ultrassonográfica, de forma a avaliar as adaptações e as respostas do tecido muscular ao aumento da atividade contrátil, usando a elastografia e a ultrassonografia em modo-B. Os estudos foram centrados no músculo do quadricípite femoral e abordaram o estudo do efeito do treino de força e da atividade contrátil muscular na espessura muscular, eco-intensidade e rigidez muscular.

Materiais e métodos: Três diferentes estudos foram realizados e descritos ao longo desta tese. Um total de 64 jovens adultos de ambos os géneros participaram dos estudos. No primeiro estudo (N = 20), foi analisada a reprodutibilidade da espessura muscular e da eco-intensidade dos quatro músculos que compõem o quadricípite femoral. Para isso foram adquiridas três imagens em modo B, nos planos longitudinal e transversal, em dois momentos distintos. A eco-intensidade foi medida usando dois tamanhos diferentes de região de interesse, um representado por uma forma retangular, medindo 70 mm^2 e um outro representando o máximo do músculo apresentado na imagem ultrassonográfica, evitando as fáscias superficial e profundas do mesmo. A precisão das medidas foi, então, analisada usando o Coeficiente de correlação intra-classe [$ICC_{(3,1)}$], o erro padrão de medição (SEM) e a menor alteração detectável (SDC). A análise de Bland-Altman foi utilizada para estudar o nível de concordância entre os planos de imagem ultrassonográficos e os diferentes tamanhos da região de interesse. No segundo estudo (N = 28), analisou-se o efeito de um programa de treino de força, com duração de 15 semanas, sobre espessura muscular e eco-intensidade em três diferentes regiões de cada um dos quatro músculos que representam o quadricípite femoral: reto femoral, vasto intermédio, vasto medial e vasto lateral. Este estudo incluiu um grupo de controlo e dois grupos de treino, em que um realizou um protocolo de treino concêntrico e o outro de treino excêntrico. Durante este estudo, as alterações na rigidez do vasto lateral, em resposta ao treino de força

foram avaliadas usando a elastografia quasi-statica, semi-quantitativa. No último estudo (N = 16), foram analisadas as alterações agudas na rigidez de vasto lateral associadas ao alongamento passivo, ao desempenho de atividade contrátil de curta duração, mas intensa e às contrações isométricas musculares usando a elastografia de onda supersónica por cisalhamento.

Resultados: Foi encontrada uma alta ou muito alta reprodutibilidade para espessura muscular (intra-sessão, ICCs: 0,82-0,99; inter-sessão, ICCs: 0,70-0,98) e eco-intensidade (intra-sessão, ICCs: 0,74-0,97; inter-sessão, ICCs: 0,48-0,94). Em geral, a reprodutibilidade para os valores da espessura muscular foi maior no plano transversal e no que diz respeito aos valores da eco-intensidade verificou-se uma melhor reprodutibilidade quando foi utilizada uma região de interesse de maiores dimensões. Um pequeno viés e menores valores de concordância caracterizam as medidas de eco-intensidade obtidas com uma região de interesse maior ou menor. No estudo 2, os participantes submetidos a 15 semanas de treino de força revelaram o aumento da sua espessura na maioria das regiões musculares avaliadas, mas não em todas. Não foram encontradas alterações significavas dos valores da eco-intensidade com a realização do treino de força na maioria dos músculos do quadricípite femoral, excepto para o vasto intermédio e para algumas regiões do vasto lateral. Por outro lado, o treino de força aumentou significativamente a rigidez do vasto lateral. Não foram observadas diferenças significativas nos parâmetros quantitativos ultrassonográficos entre o treino concêntrico e excêntrico. O último estudo demonstrou um aumento agudo de cerca de 10% nos valores da rigidez do vasto lateral como resultado da realização de contrações máximas isométricas, concêntricas e excêntricas. Os valores da rigidez do vasto lateral também aumentaram durante a flexão do joelho de 10º para 50º e posteriormente para 90º. Finalmente, observou-se uma relação linear entre os valores de rigidez do vasto lateral e o nível de contração muscular isométrica do quadricípite femoral.

Conclusões: As medidas ultrassonográficas da espessura muscular e eco-intensidade mostram uma reprodutibilidade moderada a muito alta. A reprodutibilidade e a concordância das medidas de espessura muscular e eco-intensidade são maiores no plano transversal e quando é utilizada uma região de interesse de maior dimensão. A elastografia semi-quantitativa mostrou existir um aumento significativo na rigidez muscular como resultado do treino de força. A elastografia por onda de cisalhamento supersónica é um bom método para investigar as alterações das propriedades mecânicas musculares associadas à função muscular. Estes resultados enfatizam a importância de uma avaliação objetiva e quantificável dos músculos por ultrassonografia, para estudar a adaptação muscular ao treino e função muscular, no geral.

Palavras-chave: ultrassonografia quantitativa; elastografia; treino de força; adaptação muscular.

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Con: Control group

Conc: Concentric

CT: Computed tomography

Ecc: Eccentric

EI: Echo-intensity

GCon: Concentric training group

GEcc: Eccentric training group

ICC: Intra-class correlation coefficient

LoA: Limits of agreement

MVC: Maximal voluntary contraction

MRI: Magnetic resonance imaging

MSK: Musculoskeletal

MT: Muscle thickness

QSE: Quasi-static elastography

RF: Rectus femoris

RGB: Red-green-blue

RCR: Royal College of Radiologists

ROI: Region of interest

ROM: Range of motion

SD: Standard deviation

SDC: Smallest detectable change

SEM: Standard error of measurement

SSI: Supersonic shear wave imaging

US: Ultrasound

VI: Vastus intermedius

VL: Vastus latelaris

VLL: Vastus lateralis longus

VLO: Vastus lateralis obliquus

VM: Vastus medialis

VML: Vastus medialis longus

VMO: Vastus medialis obliquus

Introduction

In the last years, the use of ultrasound (US) imaging for the study of muscle skeletal function has grown considerably. The reasons for such growth include the ability to study muscle morphology and muscle tissue mechanical properties at relatively low cost and accessibility (Wang et al., 2017). The continuous improvement of US imaging equipment, including the use of multifrequency probes, higher frequency of image acquisition, and the development of new US techniques, such as elastography have contribute to make US imaging an important method in muscle function research (Paluch et al., 2016; Walker et al., 2004).

Ultrasound imaging is a major diagnostic imaging technique, including for the clinical assessment of muscle alterations associated with neuromuscular diseases or injury (Pillen et al., 2008). Numerous studies have proven the feasibility of US imaging for studying muscle function in non-pathological conditions (Jansen et al., 2012). The assessment of parameters such as muscle thickness (MT) and texture allows studying muscle morphological responses to a variety of stimulus, in particular to muscle disuse and resistance training (Teixeira, 2013).

MT, defined as the distance between the superficial and deep fasciae of muscles, and echo-intensity (EI) are the two most important parameters of quantitative US for studying muscle adaptation due to neuromuscular diseases or demographic factors, such as age, gender, practice of sports, and sedentary lifestyle (Jansen et al., 2012; Trip et al., 2009). Exercise training can induce specific muscle adaptations depending on variables such as the type of training, and its intensity and duration (Nishihara et al., 2014; Tilp et al., 2012; Zaidman, et al., 2008). However, the results can be quite controversial, in most of the cases due to the different methodologies used. Assuring the consistency of quantitative US, in particular its reproducibility, is necessary for using US imaging and ultrasound-derived parameters in the study of muscle adaptation to physical exercise. While a number of associations have been established between MT, EI and models of muscle use and disuse (e.g., bed rest or aging), it is not clear how strength training affects those parameters in complex muscles, such as the quadriceps femoris (Strasser et al., 2013). In the case of EI, there is no consensus about how this parameter changes in response to strength training in young and healthy subjects.

A recent application of quantitative US is the evaluation of tissue stiffness. This evaluation is decisive in the early diagnosis and follow-up of a variety of diseases, including neuromuscular diseases (Pillen et al., 2008), but it is also important for the

non-invasive and dynamic assessment of muscle mechanical properties (Brandenburg et al., 2014; Yanagisawa et al., 2015). Elastography is an imaging technique that maps soft tissues' stiffness using a colour grade from which a semi-quantitative or quantitative measure of stiffness can be derived (Toledo, 2016). Using US elastography, the mechanical behaviour of muscles can be studied non-invasively and in dynamic conditions and, unlike other techniques, it can measure stiffness in localized muscle regions and in specific muscles within muscle groups (Bouillard et al., 2014; Bouillard et al., 2011; Lacourpaille et al, 2012; Nordez, et al., 2006; Toledo, 2016). Despite the fast growing number of studies employing US elastography to investigate muscle function, the effect of strength training on local muscle stiffness has not been fully explored. Also, the effect of passive stretching and muscle contraction on the amount of stiffness in complex muscles such as the Vastus Lateralis (VL) is not fully described.

Thesis aims

The overall aim of this thesis is to investigate the feasibility of ultrasound imaging for studying skeletal muscle function and adaptation to different types of contractile activity.

The specific aims of this thesis are as follow:

- To study the reproducibility of ultrasound-derived measures of muscle thickness and muscle echo-intensity for each head of the quadriceps femoris.
- To study changes in quadriceps femoris muscle thickness, muscle echo-intensity after different kinds of strength training (i.e., concentric or eccentric strength training) and different movement amplitudes.
- To study the feasibility of quasi-static ultrasound elastography for studying changes in vastus lateralis stiffness after different kinds of strength training (i.e., concentric or eccentric strength training).
- To study acute changes in vastus lateralis stiffness in response to contractile activity, passive stretching and level of isometric contraction using supersonic shear wave imaging.

The structure of this thesis is outlined in Figure 1.

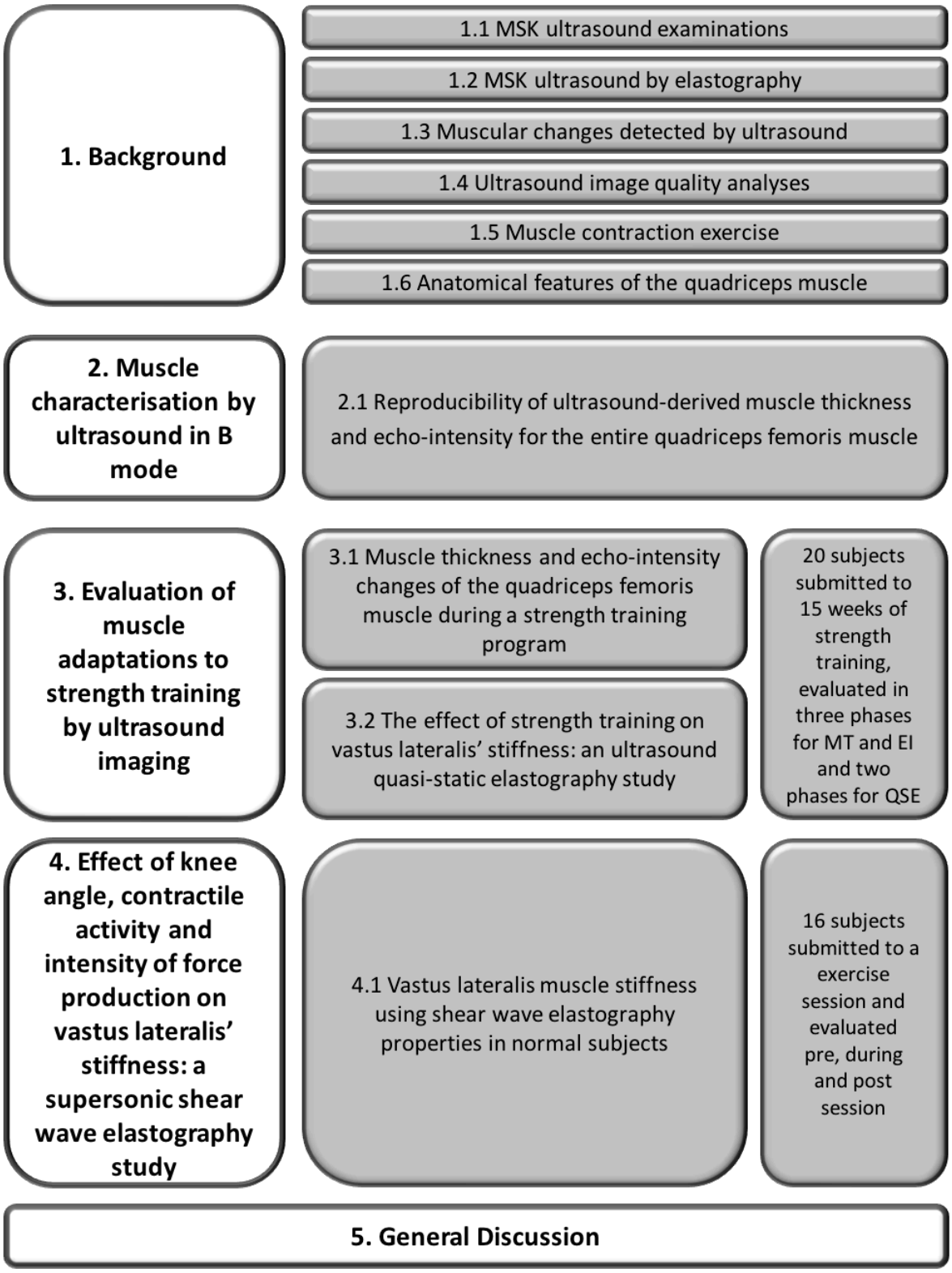


Figure 1: Thesis flowchart.

Thesis outline

This thesis is divided in five Chapters. Chapter 1 presents a literature review describing the state of the art regarding musculoskeletal (MSK) evaluation by medical imaging with a special focus on US. The major applications of US imaging and the parameters that can be quantified using this technique will be presented and discussed. A brief description of the gross anatomy of the quadriceps femoris will also be presented.

Chapter 2 reports the experimental study designed to study the intra- and inter-session reproducibility of US measures of MT and EI for each of the four heads of the quadriceps femoris in a group of healthy young adults.

Chapter 3 is divided in two sections each one describing selected parts of an interventional study of 15 weeks of strength training. The first section addresses the effect of the 15-week strength training program on MT and EI in several regions of the heads of the quadriceps femoris. In the second section, the effect of the same strength training program on VL's elasticity, measured by means of quasi-static elastography (QSE) is reported.

Chapter 4 reports the last experimental study of this thesis. In this preliminary study, SSI was used to investigate acute changes in VL's stiffness caused by passive stretching, intense contractile activity, and isometric contraction.

Chapter 5 presents a general discussion addressing the main findings in the thesis, major limitations, and recommendations for future research.

Chapter 1: Background

Medical imaging plays nowadays a central role in the diagnosis and follow-up of MSK diseases. Ultrasound imaging, together with magnetic resonance imaging (MRI), have increasing importance in this area due to their evolving ability for soft tissue evaluation. On the other hand, general radiography shows many limitations for evaluating soft tissues and exposes the patients to ionizing radiation (Tan et al., 2003).

The latest edition of iRefer guidelines of the Royal College of Radiologists (RCR) (2012) has a chapter fully dedicated to MSK pathology and identifies 27 MSK clinical problems to support clinicians in making appropriate referral decisions (RCR, 2012). A recent RCR member stated that new European referral guidelines for radiological imaging must be promoted to support the good practice in medical imaging prescription (Remedios et al., 2014). For the majority of these clinical situations, US imaging is indicated for a medical assessment taking into account its accuracy and non-exposure to ionising radiation (RCR, 2012).

Advantages and disadvantages of medical imaging examinations for MSK studies can be compared in Table1.

Table 1: Comparison of specific imaging techniques (adapted from: Boykin et al. 2010)

Modality	Pro	Contra	Risk	Cost
Radiography	Good evaluation of bone.	Unable to evaluate soft tissue.	Low radiation exposure.	Low cost.
US	Good visualization of soft tissues; Dynamic testing possible; Low costs; Availability.	Unable to evaluate deep joint structures (e.g. shoulder); Highly dependent on examiner skills.	No identifiable risk.	Low to medium cost.
MRI	Good visualization of soft tissues; Good visualization of concomitant injuries such as labrial tears; Good operative planning possible.	Possible false positive results; Availability.	Potential risk of nephrogenic systemic fibrosis in renal insufficiency.	High cost.
MR-arthrography	Superior to conventional MRI and US in respect of sensitivity and specificity of detection of soft tissue tears (also see MRI).	Invasiveness; Availability.	————	High cost.

Computed tomography (CT)	Good evaluation of bony defects.	No good visualization of soft tissue; Availability.	Moderate radiation exposure.	Medium cost.
CT-arthrography	Good evaluation of bones combined with reasonable evaluation of soft tissues.	Invasiveness Availability.	Moderate radiation exposure.	Medium cost.

Ultrasound diagnostic for injuries, inflammation or chronic problems is reliable and increasingly common. Some specific structures are more affected, but pathological changes are similar in the tendons, ligaments, and muscles regardless their location (Salh, 2015). The evaluation of tendon diseases or injuries is probably the most common clinical indication for US evaluation. This method has a high sensitivity (100%) for full thickness tears but, similar to MRI, has a much lower sensitivity for partial thickness tears (Lento & Primack, 2008).

Compared to US, general radiology and MRI are useful for evaluating intra-articular and peri-articular alterations, however US can add some information like detecting small joint effusions, and helping on its location as a guide for aspiration (Lento & Primack, 2008).

Ultrasound examination includes the ability to perform dynamic imaging, as well with sono-palpation or motion. With US, tendon subluxation or dislocation can be visualized with dynamic manoeuvres. The same cannot be performed with MRI. Abnormalities like tendon clicks and snaps or impingement syndromes are easily evaluated by US (Lento & Primack, 2008; McNally, 2011).

MSK ultrasound is a good method to clearly define the extent of an injury and to detect the cause of the underlying effusion. With Doppler US, the synovium of inflammatory or infectious arthritis can be evaluated. Ultrasound evaluation was introduced on the peripheral nervous system for the diagnostic of carpal tunnel syndrome (Lento & Primack, 2008).

Due to its portability, user friendly, and superior spatial resolution US is considered an excellent imaging modality for detecting and classifying a large number of MSK injuries. This method can also identify non-traumatic or primary muscle pathologies such as myositis and it can diagnose more rapidly muscle sports injuries (Lento & Primack, 2008). Ultrasound allows the distinction between different grades of muscle strain (Lee & Healy, 2004).

Ultrasound has become a well-established method in the evaluation of sports-related injuries of both the upper and lower extremities. Its accuracy has been confirmed for many types of diseases (Blankenbaker & De Smet, 2006; Lento & Primack, 2008).

Around 10-55% of all sport injuries are muscle injuries and it is estimated that over 90% of sports related injuries are strains or contusions. Muscle strain injuries can result from a faulty contraction or from excessive stretching (Alqahtani, 2010).

1.1 MSK ultrasound examination

Ultrasound image has improved over the past few decades, increasing its clinical application (Lee & Healy, 2004). In the last years the use of MSK ultrasound had significantly increased with a 3-fold increase in the number of studies performed between 2000 and 2009 (Petscavage-Thomas, 2014). Salh (2005) argues that MSK ultrasound should be the first examination for most pathological conditions and it should be done for every patient complaining of swelling, pain and trauma before doing general radiology or MRI (Salh, 2015). Ultrasound is an excellent imaging modality for most MSK problems, allowing the evaluation of various structures including tendon, muscle, joints, even nerve and some osseous pathology with excellent resolution (Lento & Primack, 2008).

Comparing with fluoroscopy and CT methods, US offers advantages when used for interventional procedures. Although fluoroscopy or CT scan can be helpful in the localization of the structure to be targeted, both require ionizing radiation. In addition, fluoroscopy does not allow soft tissue visualization, relying on bony landmarks and often use contrasting agents in its procedures (Lento & Primack, 2008).

One important advantage of US imaging for MSK evaluation is that it allows patients to move during examinations and therefore is capable of supporting the diagnosis of several pathological conditions that are elicited only through patient movement (Lee & Healy, 2004). Ultrasound has also some advantages over MRI, which includes accessibility, lower cost, and more patient friendly. It allows a more direct imaging correlation with patient symptoms, which provides important information (Blankenbaker & De Smet, 2006; Lento & Primack, 2008). However, MRI allows a larger area to be examined but this is not always an advantage since several ‘‘abnormalities’’ may be detected that may be clinically unrelated to the patient’s complaints. On the other hand, US with the application of extended field of view imaging can also examine large areas while preserving the interaction with the patient. Ultrasound permits a real time imaging, observing pathologic movement in tendon, bursa, muscles, or joints, while in

MRI movement distorts image quality and introduces artefacts (Lento & Primack, 2008; McNally, 2011).

The US transducers are used according to the type of structures to be visualized (Fulton, 2014; Lento & Primack, 2008). The choice of the transducer should be made based on the type of examination, the organ evaluated, and the patient's biotype. There are at least five types of transducer, however only the linear transducer will be mentioned because it is used most of the times to visualise MSK structures. The linear transducer performs a linear scan (it has the shape of a rectangle) and the frequency ranges from 7 to 18 MHz. It is used in examinations of superficial structures, such as breast, thyroid, MSK system and peripheral vascular exams. The field of view is directly proportional to the width of the transducer. The use of the appropriate transducer frequency for the structure to be evaluated is extremely important (Fulton, 2014). The higher the frequency of the transducer, the higher is the resolution of the image and the lower is the depth reached (Hammond et al., 2014).

1.1.1 Advantages and disadvantages of diagnostic US in MSK

Ultrasound imaging does not use ionizing radiation and contrasts, when used, do not cause known adverse reactions. It is used as a guide for interventional procedures, as aspirations or drainages (Lento & Primack, 2008). Portability allows examination not only in the workplace but also in the training room and playing field (Lento & Primack, 2008). The real-time capability US allows dynamic evaluation of muscle and tendon injuries (Lee & Healy, 2004). High-frequency transducers yield images with excellent spatial resolution and this is particularly useful for MSK imaging (Lento & Primack, 2008). Recent advances in tissue harmonics have improved visualization and resolution of deeper structures even in obese patients (Lento & Primack, 2008).

One limitation of US imaging is its dependence on body habitus. Ultrasound wave penetrance into tissue is inversely proportional to the wave frequency. The anisotropy artefact can be another disadvantage, affecting US diagnosis ability because it can mimic real pathology (Lento & Primack, 2008). Another limitation of US diagnosis includes operator dependence, that can be overcome with training and experience (Blankenbaker & De Smet, 2006). MSK ultrasound should be done by skilled examiners with knowledge about anatomy, physiology and pathology and they should be very familiar with US equipment in order to produce images of high quality (Whittaker & Stokes, 2011). Despite these disadvantages, US imaging has been the method of choice for the diagnosis of muscle injuries (Lee & Healy, 2004; Lento & Primack, 2008). The advantages and disadvantages of US in imaging muscles are presented in Table 2.

Table 2: Advantages and disadvantages of applying US in MSK imaging.

Advantages of US	Disadvantages of US
✓ Non-invasive examination. ✓ No use of ionising radiation or contrast reagents. ✓ Good and excellent soft resolution contrast. ✓ High specificity and sensitivity values. ✓ Pathology treatment follow-up. ✓ Portable and less expensive procedure. ✓ Useful in the evaluation of muscle trauma.	✓ Operator dependent. ✓ Lower special resolution than MRI and CT scans.

1.1.2 Parameters of B mode ultrasound evaluation

Several parameters are taken into account when structures are assessed by US, being the following the most used: echo-intensity, echo-structure, contour and dimensions (namely thickness). Ultrasonography is used to measure morphological muscle-tendon alterations, including changes in thickness and EI. As such, these parameters are being increasingly analysed and associated with muscle function and muscle mechanics.

Muscle thickness (Figure 2) is defined as the distance between the most superficial aponeurosis and the deepest aponeurosis of the muscle (Delaney et al., 2010; Teixeira, 2013; Verhulst et al., 2011). It is a quantitative parameter obtained in both transversal and longitudinal images to assess muscle chronic adaptations to different strength training protocols and it is associated with muscle strength capacity (Radaelli et al., 2011). Studies reveal that the accuracy of MT as a predictor of muscle strength is relatively low comparatively with measurements of muscles' cross-sectional area (Muraki et al., 2013). Yet, some authors support the use of MT as a predictor of the muscles' cross-sectional area (Muraki et al., 2013).

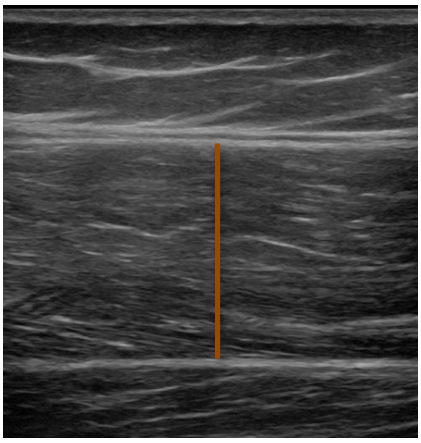


Figure 2: B mode ultrasound image of the vastus lateralis and of its thickness measured by Image J software. Muscle thickness was determined as the distance between superficial fascia of the muscle and the deep fascia of the same muscle.

Echo-intensity, assessed by ultrasonography, is the capacity of a tissue to reflect the US waves and produce echo, and it may contain information about the muscle tissue status. The fact that US beams penetrate easily through the majority of structures leads to structures appearing as hypoechoic (this happens with liquids and with low density tissue). When the contrary occurs, i.e., when the US beams have more difficulty in passing through the structures, there is higher reflection of the US and the tissues appear as hyperechoic (connective tissue and fat)(Wilhelm et al., 2014).

Echo-intensity thus allows to assess alterations in the quantity of the non-contractile intramuscular components (Nielsen et al., 2006; Wilhelm et al., 2014), caused by muscle pathology or simply by degenerative alterations associated with ageing (Pillen et al., 2009).

In the past, and still today during clinical practice, EI is assessed in a qualitative manner, depending on a visual analysis done by the operator, who relies on his experience to reach a conclusion or a clinical decision. For this reason, this method is subjective and insensitive to small alterations. Currently, however, most studies evaluate EI quantitatively based on a grayscale analysis (Fukumoto et al., 2012; Pillen, 2010; Pillen et al., 2009). This technique consists in assessing the distribution of gray levels in the image and its variation, i.e., it is based on the assessment of the intensity of gray levels across the image (Alqahtani, 2010). This quantitative EI analysis is more reliable and it is less subjective, providing information that goes beyond the mere visual interpretation of the image's EI pattern (Fukumoto et al., 2012; Ríos-Díaz et al., 2010).

This quantitative method requires simple software for image edition but it is sensitive to differences in hardware and software between US machines (Pillen et al., 2009). All system configuration parameters cannot be changed during image collection and depth must be set taking into account the visualisation of the structure under study and the overall gains and dynamic range value. The time gain compensation must be kept uniform and the angle of the transducer must be perpendicular to the assessed tissue (Alqahtani, 2010).

A healthy muscle is visualised in an US image as hypoechoic, i.e., with a low EI value, due to the small amount of non-contractile tissues (Pillen et al., 2009; Teixeira, 2013). In general, a diseased muscle, a muscle suffering from inactivity or a muscle from an aged person displays higher EI levels, i.e., it becomes more hyperechoic and more diffuse compared with the muscles of healthy young subjects (Pillen et al., 2009). In contrast to the muscle belly, tendons are hyperechoic and become hypoechoic with ageing (Nielsen et al., 2006).

The B-mode US image is a combination of pixels displaying a specific intensity of gray (Ríos-Díaz et al., 2010). Upon editing the image, the researcher should select the area of interest in the muscle, and the software calculates the different levels of gray existing in that selected region of interest (ROI) (Pillen et al., 2009). The ROI, which is visually selected and should include as much of the target muscle as possible, avoiding the surrounding bone or fascia, may include the cross sectional area of the muscle (Caresio et al., 2015). Being aware of the typical pattern that is characteristic of the studied muscle is important, as the hyperechoic pattern of the image, such as the presence of internal aponeurosis (e.g., the RF muscle) or the heterogeneous distribution of the EI may skew the distribution of gray level values obtained for the entire muscle cross-section (Caresio et al., 2015).

Quantitative measurements of EI is a relatively recent method of ultrasonography imaging which has raised the interest of several authors, namely for MSK studies. This method has been associated with the assessment of tendinous and muscle morphological alterations caused by pathologies or lesions, often being associated with adipose infiltration of connective tissue and/or interstitial oedema (Alqahtani, 2010; Radaelli et al., 2012; Ríos-Díaz et al., 2010). These tissue alterations increase the US beam reflection and result in an increase of the EI, which may display a specific spatial distribution within the muscle (Caresio et al., 2015; Pillen et al., 2008). The quantification of EI (Figure 3) may also be used in the characterisation and differentiation of muscle structures among athletes and sedentary individuals, as well

as in the analysis of alterations caused by muscle tiredness and fatigue (Alqahtani, 2010; Caresio et al., 2015).

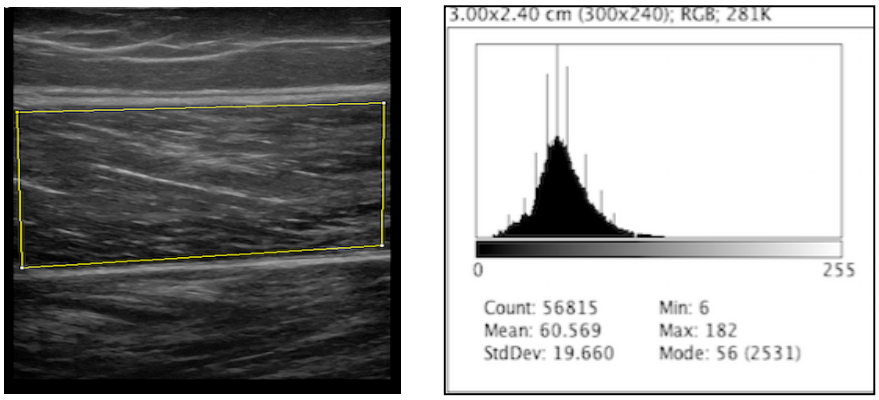


Figure 3: Echo-intensity measurement. (A) B mode ultrasound of the vastus lateralis with a maximum ROI selected; (B) Histogram of the pixel graylevel values distribution and statistics obtained using Image J software.

1.2 MSK ultrasound by elastography

Elastography is a non-invasive technique that allows the characterisation of the mechanical properties of the tissues, aiming to determine the respective Young' modulus or the amount of deformation that the tissue suffers when a load is applied (Vega, 2011). This technique has been evolving rapidly, demonstrating great potential not only in the diagnosis of diseases characterised by alterations of tissues' stiffness, but also for the physiological and morphological study of different structures/tissues (Cosgrove et al., 2013).

Elastography started off by being a qualitative and/or semi-quantitative technique, but evolved towards a quantitative by offering elasticity maps with the use of conventional ultrasonography equipment in real time, by means of software developments (Gheorghe et al., 2009; Zordo et al., 2009). Ultrasound elastography was described for the first time by Ophir et al. in 1991 (Konofagou et al., 2003; Ophir, 2005) and, later on, it evolved into an imaging tool in real time (Ophir et al., 1997). It may be defined as a dynamic technique developed to offer an estimated value of the elasticity/rigidity of the tissue, measuring the degree of its distortion when subjected to an external force (Pedersen et al., 2012). Ultrasound elastography is a complementary technique to the B-mode US, offering high diagnosis sensitiveness regarding the detection and assessment of the nature and structure of pathologic alterations in the body (Castaneda et al.,

2010; Smajlovic et al., 2011). Several studies show that it is a reliable technique for the diagnosis of hematoma, oedema, fibrosis, benign and malignant solid lesions, allowing the early detection of neoplasms (Abella & Zordo, 2008; Castaneda et al., 2010; D'Onofrio et al., 2014; Fierbinteanu-Braticevici et al., 2012; Goddi et al., 2011; Iglesias-Garcia et al., 2009; Lee, 2009). Due to its advantages and indications, US elastography tends to be part of every ultrasonography exam (Castaneda et al., 2010; Smajlovic et al., 2011).

This first studies employing US elastography were carried out for the diagnosis of breast, thyroid and prostate neoplasms (Rizzatto, 2008). Later on, advances in this method made possible to study deeper structures, such as the liver and pancreas (D'Onofrio et al., 2014; Kudo et al., 2013; Pedersen et al., 2012).

Ultrasound elastography has also great potential for the diagnosis of MSK diseases (Abella & Zordo, 2008; Monetti & Minafra, 2007). Because the mechanical properties of tissues are usually altered after injury or disease (e.g., inflammation, ageing and malignancy), by using US elastography tissue abnormalities may easily be identified (Pedersen et al., 2012).

Elastography, including US elastography, started being used in MSK biomechanics research soon after its development. Both tension and shear wave elastography may be used in the study of muscle-tendon structures (Figure 4). Although most authors use tension elastography, more and more studies appear using shear wave elastography.

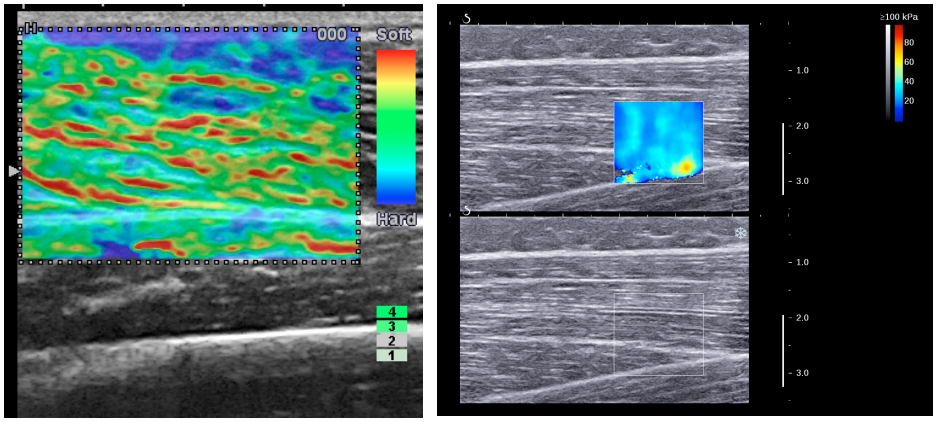


Figure 4: Elastography by ultrasound imaging. (A) Quasi-static elastography; (B) shear wave elastography.

The type of elastography depends on the method of stress application and their objectives. It includes compression elastography, shear-wave elastography, and

transient elastography. Each one has advantages, artefacts, limitations and specific clinical applications (Klauser et al., 2014).

However, all types of elastography operate following three steps: stress or distortion to the ROI; the tissue response (strain) and processing the distortion (Toledo, 2016). The differences between different elastography methods also reside on how the distortion is applied to the tissue and on the type of force that is applied.

1.2.1 Physical principles of elastography

Assuming that tissues are elastic (i.e., they return to their initial shape after undergoing deformation), isotropic (their elastic modulus does not depend on the orientation of the tissue), incompressible (no volumetric variations when deformed), and homogeneous, there exists four fundamental modulus that can be associated with each other: modulus of elasticity or Young's modulus, shear modulus, volumetric modulus and Poisson's ratio (Cavalcanti, 2012; Vega, 2011).

The elastic modulus or Young's modulus is a mechanical parameter proportional to the rigidity of a solid structure when subjected to an external tension or compression (Cavalcanti, 2012; Gennisson et al., 2013; Rizzatto, 2008). The shear modulus is based on the sliding of planes parallel to each other when forces are applied in parallel (Cavalcanti, 2012; Gennisson et al., 2013). The volumetric module measures the tendency of a tissue to deform in all directions when applying a multidirectional force (Gennisson et al., 2013; Smajlovic et al., 2011; Vega, 2011). Poisson ratio measures transverse deformation of a tissue when a longitudinal force is applied (Cavalcanti, 2012; Gennisson et al., 2013).

The basic principle of elastography is that stress applied to tissue causes changes within it, which depends on its elastic properties. Elastography then evaluates tissues' elasticity by taking into account their deformation when a force is applied (Cosgrove et al., 2013; Drakonaki et al., 2012; Smajlovic et al., 2011).

Since its emergence, different generations of elastography have been developed, depending on the type of stress application and the method used to detect tissue displacement and obtain the image. However, all types of elastography use force and measure the deformation produced by this force on the tissue (Cosgrove et al., 2013; Drakonaki et al., 2012; Smajlovic et al., 2011).

1.2.2 Methods of elastogram interpretation and analysis

The methods of elastography can be divided into three broad groups: qualitative, semi-quantitative, and quantitative (Cosgrove et al., 2013; Franchi-Abella et al., 2013; Toledo, 2016). All these elastography methods produce a colour map, which is known as an elastogram (Toledo, 2016). These elastograms are available in most equipment, regardless of the type of elastography used (Franchi-Abella et al., 2013). The elastogram is generated by software and is usually depicted as a semi-transparent overlay of the grayscale US image (Toledo, 2016). They can be used as grayscale or colour scale depending of the US manufacturer (Franchi-Abella et al., 2013). When a colour-coded elastogram is used, usually the blue colour is chosen for hard tissue, red for soft tissue and green for intermediate stiffness (Barr et al., 2015; Toledo, 2016).

The qualitative evaluation is obtained from visual inspection of the elastogram (Franchi-Abella et al., 2013). The big disadvantage of this type of elastography is its reliance on the operator and poor reliability (Pochini et al., 2015; Toledo, 2016).

Regarding semi-quantitative elastography, two methods are available: strain-ratio, and histogram of pixel distribution. The first one gives a strain index or elasticity ratio between two regions of interest (Franchi-Abella et al., 2013). The second method, is based on measuring the number of pixels of a given colour within a ROI (Toledo, 2016). This analysis can only be done after the acquisition of the image and by using another software, like Image J, Matlab, or any other comparable software (Toledo, 2016). This type of analysis is less operator dependent, has a higher reliability and provides an indirect stiffness value (Toledo, 2016).

Quantitative US elastography is available from shear wave propagation velocity measurement techniques. In this case, elastography uses measurements of the wave speed travelling through the tissues. The elastogram now gives a quantitative measurement of tissue's stiffness in the form of the modulus of elasticity, expressed in kilopascal (kPa) (Franchi-Abella et al., 2013; Toledo, 2016). Therefore, shear wave elastography provides a direct measure of tissues' stiffness (Toledo, 2016).

Elastography artefacts

Despite the great improvements in elastography, there are some important artefacts associated with this technique (Cosgrove et al., 2013; Franchi-Abella et al., 2013). For example, force-strain relationship is non-linear and time dependent, the elasticity varies spatially and with direction, the contours and the structure of the tissues can alter the relationship between the shear wave velocity and the shear modulus, and

tissues can be discontinued mechanically by anatomical features, such as tumours or scars (Cosgrove et al., 2013). It is important to be familiar with the pitfalls and artefacts of US elastography for a correct interpretation of the elastograms.

1.2.3 Methods for force application

Two types of elastography can be considered regarding the method used to apply force: quasi-static and dynamic.

Quasi-static elastography

In quasi-static elastography (QSE), stress is applied upon the tissue by an external vibration applied with the transducer (Cosgrove et al., 2013; Gennisson et al., 2013; Toledo, 2016). The obtained images are semi-quantitative and do not directly describe the elasticity of the tissue since the amount of tension produced within the tissue is unknown. However, ROIs can be drawn in the area under study and in a reference region in order to calculate the ratio and obtain a semi-quantitative analysis (Cosgrove et al., 2013; Toledo, 2016).

Together with absence of true quantification, a major limitation of QSE is still the lack of control over the applied force. Moreover, the use of operator-imposed pressure limits the study to surface structures (Gennisson et al., 2013).

Dynamic elastography

In dynamic elastography, the force or source of stress is generated by the US probe (Toledo, 2016). The force applied can be a time-varying force, a short transient mechanical force, or an oscillatory force with a fixed frequency (Gennisson et al., 2013). This method of US elastography has the advantage of being quantitative and also of not depending on the operator or on an external actuator to produce the stress (Cosgrove et al., 2013).

1.2.4 Elastography techniques

The main elastography techniques used are strain elastography, acoustic radiation force impulse elastography, transient elastography, and shear wave elastography (Figure 5).

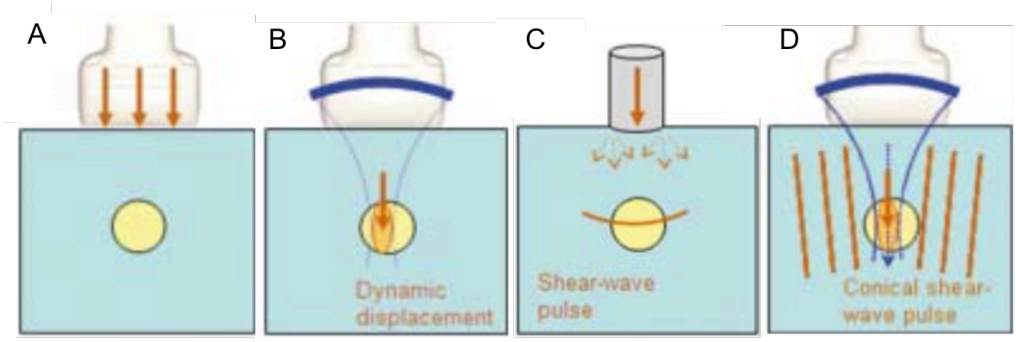


Figure 5: Scheme of ultrasound elastography methods. (A) Strain elastography; (B) acoustic radiation force impulse elastography; (C) transient elastography; (D) shear wave elastography. (Adapted from Bamber et al., 2013).

Strain or compression elastography

Strain elastography, also described as compression elastography, is based on the quasi-static method and is a qualitative or semi-quantitative analysis (Gennisson et al., 2013; Klauser et al., 2014). The quantified parameter is strain, which is produced by repeated manual pressure of the tissue under investigation (Kim et al, 2015; Klauser et al., 2014). The difference in the echo produced by the pressure/strain is calculated (Modulus of elasticity=stress/strain), measuring the relative strain of one area compared to that of another and the results are represented by a colour-coded strain distribution map (elastogram), which is often superimposed over the conventional B-mode image or displayed next to it (Kim et al., 2015; Klauser et al., 2014). The elastogram is adjustable by the user and this technique can draw the calculation area as a relatively free shape (Kim et al., 2015; Klauser et al., 2014). Most compression elastography equipment provide visual information about the applied pressure on the screen (Kim et al., 2015).

Acoustic radiation force impulse elastography

In this type of elastography, the tissues are deformed by US pulses that are produced by a focused radiation force. The tissues' displacement can be measured using several short-time pulse echoes and then comparing the results with the reference image (Barr et al., 2015; Klauser et al., 2014). This technique is based on qualitative analysis and the results are represented by a colour-coded or grayscale elastogram. This technique can evaluate deeper tissues (Cosgrove et al., 2013).

Transient elastography

Transient elastography, also designed by pulsed elastography, uses a controlled external tone burst of vibration to generate shear waves (Barr et al., 2015; Klauser et al., 2014). This technique can provide quantitative evaluation, measuring the average shear wave

velocity within a ROI converted to Young's modulus, expressed in kPa (Barr et al., 2015; Drakonaki et al., 2012; Klauser et al., 2014). Transient elastography provides only regional elasticity measurement with limited depth and is mainly used for liver studies (Drakonaki et al., 2012; Klauser et al., 2014).

Shear-wave elastography

Shear-wave elastography is a dynamic method and it is based on measuring the propagation velocity distribution of the directional shear wave, produced by an US pulse (Drakonaki et al., 2012; Klauser et al., 2014). The velocity of the shear waves can be measured and used to evaluate tissue elasticity by the Young's modulus (E) calculated by the formula $E = 3rV^2$ (E = Young's modulus; V = shear wave velocity; r = material density) (Klauser et al., 2014). This technique provides both qualitative elastograms and quantitative measurements, which are presented in quantitative maps with units in kPa (stiffness) or in centimetres per second (shear wave velocity) (Drakonaki et al., 2012; Klauser et al., 2014). Shear wave elastography has a depth limitation and only limited ROI shapes are available for the quantitative measurement of elasticity (Klauser et al., 2014).

1.3 Muscular changes detected by ultrasound

In an US image, the muscle tissue features different characteristics and it can be easily distinguished from the surrounding structures, such as adipose and subcutaneous tissue, bone, nerves and blood vessels (Alqahtani, 2010; Pillen, 2010).

The healthy muscle presents low echo density (i.e. it is hypoechoic) (Pillen, 2010). The few echoes generated are due to the existence of minimal interfaces available for US reflection, caused by the muscle cells which are composed by an internal, highly organised cytoplasm structure, and by connections of identical structural proteins (Alqahtani, 2010; Walker et al., 2004).

In the transversal plane, perpendicular to the muscle longitudinal axis, the muscle has a poor echogenic appearance, although somewhat heterogeneous, due to the presence of countless hyperechoic, curvilinear and dotted reflections of the perimysial connective tissue involving the muscle fascicles and also of adipose tissue (Alqahtani, 2010; Pillen, 2010; Vlychou & Teh, 2008; Walker et al., 2004).

In the longitudinal plane (along the muscle fascicles' axis) the fascicular architecture of the muscle becomes visible. The reflections of the perimysial connective tissue are now visible as linear echogenic structures similar to septa (Lee & Healy, 2004; Pillen, 2010;

Walker et al., 2004). These tissues of the perimysium vary in thickness and intensity along the muscle (Alqahtani, 2010; Walker et al., 2004).

The muscle limits are easily identified through the epimysium surrounding the whole muscle, which displays high EI (Verhulst et al., 2011). In other words, when the US beams encounter an acoustic surface, for example in the transition of the muscle and the epimysium, a significant portion of the US is reflected (Pillen, 2010).

On the other hand, the acoustic impedance between the muscle and the bone is very different, causing a strong reflection, defining the external limit of the bone with high EI, accompanied by a characteristic bone shade, preventing the US from penetrating into the deepest structures (Pillen, 2010; Walker et al., 2004). This typical image of the bone is useful in comparative studies using the opposite extremity, or in longitudinal studies (Walker et al., 2004).

In muscles such as the RF, the most prominent echo is generated by its peripheral aponeurosis (Alqahtani, 2010; Vlychou & Teh, 2008; Walker et al., 2004). This aponeurosis contains collagen fibres randomly distributed and has high EI so it can be structurally (and acoustically) distinguished from the highly-organised muscular architecture (Alqahtani, 2010; Walker et al., 2004).

The subcutaneous adipose tissue has low EI. However, in its inner part it presents several eco-intense septa of connective tissue (Alqahtani, 2010; Pillen, 2010).

The nerves and tendons present a relatively high EI when compared with healthy muscles, while blood vessels are characterised by being circles (transverse plane) or lines (longitudinal plane) - hypo or anechoic – and their presence may be confirmed with the use of US Doppler to demonstrate the presence of blood flow (Pillen, 2010).

The superficial muscles may easily be seen through ultrasonography. The advances in technology also allow a better resolution, making it possible to identify small individual muscles even when they are overlapped by other muscle groups (for example, in the hand). Deeper muscles (for example major psoas or paravertebral muscles complexes) may be difficult to visualise with sufficient resolution with US, due to the reflection or absorption of the US beams by layers of superficial tissues, such as the skin, the subcutaneous tissue or other muscles (Pillen, 2010; Walker et al., 2004). In this case, convex transducers are often used.

Each skeletal muscle has different proportions of perimysial tissue and, therefore, the level of EI changes with different muscles (Alqahtani, 2010; Walker et al., 2004). On the other hand, the muscles may show a considerable variation in the fascicular

arrangement regarding the tendons to which they are connected (Vlychou & Teh, 2008). Muscles may be unipennate (hamstring muscles), bipennate (biceps muscle), with peripheral aponeurosis (RF), or central aponeurosis (tibialis anterior muscle) (Alqahtani, 2010; Vlychou & Teh, 2008; Walker et al., 2004). The layout of the fascicles is intimately connected to the relative strength and muscle movement amplitude.

The structures that compose the quadriceps femoris show important differences as far as their visualisation in the ultrasonography is concerned that result from differences in shape, size, depth, morphology, and tissue composition (Kinugasa et al., 2005). The proximal tendons of the RF muscle present regular, smooth and homogenous hyperechoic contours. The RF muscle has a typical aponeurosis which has been previously described, shaped like a comma, hyperechoic, which stands out in the muscle tissue (Pasta et al., 2010). The vasti muscles possess small septa of perimysial tissue. The three vasti can be distinguished by the hyperechoic fascia surrounding each of them (Pasta et al., 2010).

The first studies in MSK evaluation were limited to compare the normal and pathologic tissue using strain elastography. With the appearance of the dynamic elastography and quantitative evaluation, many studies were performed to characterise the normal muscle and its changes with contraction, stretching or ageing (Andonian et al., 2016; Drakonaki et al., 2012; Hirono et al., 2016; Klauser et al., 2014; Pochini et al., 2015). In a qualitative view, the muscle at rest is seen as an inhomogeneous mosaic of intermediate or increased stiffness with scattered softer and harder areas, especially at the periphery near boundaries (Drakonaki et al., 2012). On the other hand, with a quantitative evaluation some muscles were already characterized during passive stretching (Xu et al., 2015) or after physical exercise (Hirono et al., 2016).

The reliability and validity of US elastography for measuring absolute muscle hardness/stiffness has been studied (Chino et al., 2012) and it is a potentially interesting technique to apply in clinical research projects that need an accurate assessment of muscle mechanical properties (Drakonaki et al., 2012; Klauser et al., 2014; Lacourpaille et al., 2012).

A study by Lacourpaille et al. (2012), where elastic modulus for several muscles was measured by US elastography by chiselling with ultrafast imaging, revealed that the elastic modulus varied between 2.99 kPa and 4.50 kPa (Lacourpaille et al., 2012). This variability in stiffness values among the different muscles is due to factors connected with the mode of acquisition and measuring and the type of muscle. The measuring of the chiselling elastic modulus proved to be dependent of the length of the muscle. On

the other hand, Toursel et al. (2002) mentions that the stiffness of the muscle is connected with the fibre typology (Toursel et al., 2002).

The percentages of adipose tissue and collagen infiltration should also influence the chiselling elastic modulus, thus causing changes in stiffness values in different age groups. It is important to take into account also the influence of probe orientation, due to muscle anisotropy (Gennisson et al., 2010; Lacourpaille et al., 2012). Gennisson et al. (2010) has shown that the penetration angle of muscle fascicles also contributes for the differences in values of the elastic modulus in the different muscles, which is why it should be performed along the direction of the fascicles (Gennisson et al., 2010).

Andonian et al. (2016) concluded that using the elastography it is possible to observe a decrease in quadriceps femoris stiffness, after a very demanding physical offered (Andonian et al., 2016).

1.4 Ultrasound image quality analysis

1.4.1 Optimisation of ultrasound image acquisition

The US image can be optimised changing the depth, general gains, time gain compensation, frequency, focus, and the dynamic range (Figure 6) (Fulton, 2014; Zaidman et al., 2008).

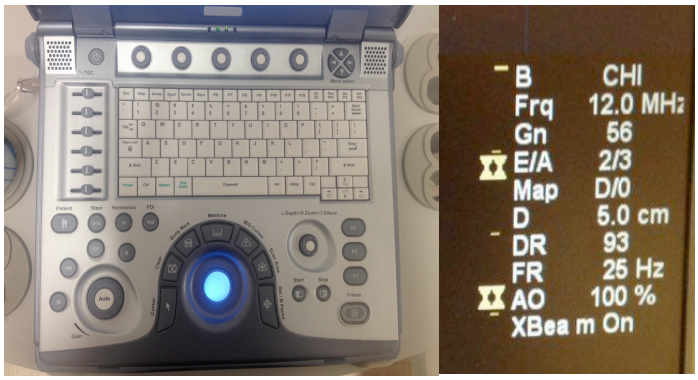


Figure 6: Example of ultrasound image parameters that can be manipulated to image optimisation. On the right side the display of this settings (frequency: 12 MHz; gain: 56; depth: 5.0 cm; dynamic range: 93). (Picture of LOGIQe equipment, General Electric Healthcare, GE Ultraschall, Deutschland GmbH & Co, Germany.)

These parameters influence the image quality in terms of spatial, temporal and contrast resolution and can also increase or decrease the artefacts appearance (Rumack et al., 2014).

In US imaging, the spatial resolution must be analysed on axial and lateral directions. The axial resolution is the resolution along the axis of the US beam. This resolution is defined as the capacity to distinguish two objects parallel to the US beam and is determined by the length of the pulse. Therefore, the higher the frequency of the transducer, the better the axial resolution of the image will be and, consequently, the distinction of detail in the depth will be lower. The lateral resolution is the resolution perpendicularly to the US beam and parallel to the transducer (Neves, 2002; Rumack et al., 2014). In other words, lateral resolution designates the ability to differentiating structures that are perpendicular to the axis of the beam. Lateral resolution is inversely proportional to the width of the beam and it depends on the number and density of US waves sent on and the echoes received (Neves, 2007; Rumack et al., 2014). The higher the frequency of the transducer, the narrower will be the beam and the better the lateral resolution will be.

During an US examination, several artefacts may occur that blunt images' quality. Artefacts are related with physics imaging principles and the most frequent are resolution, propagation, attenuation and anisotropy artefacts (Fulton, 2014; Pasta et al., 2010; Walker et al., 2004).

Anisotropy artefact is common in MSK ultrasound (Figure 7). It depends on the angle between the US beam and the tissues and it occurs when the US beam is not perpendicular to the imaged structure (Lento & Primack, 2008). A smaller number of US waves are reflected and, consequently, detected by the transducer leading to a reduction in the EI (brightness) of the tissue being examined (Lento & Primack, 2008; Pasta et al., 2010; Walker et al., 2004).

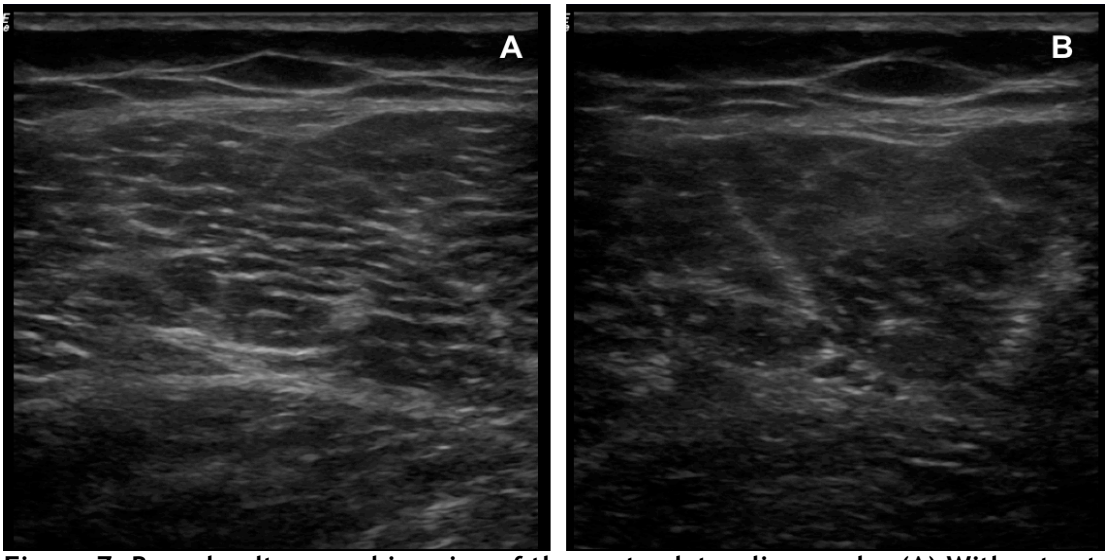


Figure 7: B mode ultrasound imaging of the vastus lateralis muscle. (A) Without artefact; (B) with anisotropy artefact.

In B mode US, images are analysed mostly based on subjective opinion (operator). Although a few parameters can be quantified with US imaging, during daily practice usually only the thickness is measured (objective evaluation). The subjective image analysis should be performed by trained observers (Håkansson et al., 2010; Ledenius et al., 2010)

When QSE is performed, the stiffness is quantified usually with the purpose of identifying some pathology. ROI's of normal and abnormal tissues are draw to analyse the colour scale, RGB pixel values, and pixel ratio differences. In wave elastography the system automatically gives a colour scale and stiffness quantification (kPa) per tissue. However, the elastography colour scale analysis is still a subjective opinion.

As already mentioned, US imaging might be employed for evaluation of the so-called muscle quality through measured of EI, MT, and stiffness, which have been used to diagnose muscle abnormalities and to identify muscle physiological adaptations caused by physical exercise (Harris-Love et al., 2014; Pillen et al., 2006; Whittaker & Stokes, 2011). Such US parameters have demonstrated value in the assessment of neuromuscular diseases and may be an important source of information regarding muscle performance (Fukumoto et al., 2012; Harris-Love et al., 2014; Zaidman et al., 2010).

The thickness of the muscle is easily obtained with the inbuilt measurement system of the US equipment. Ultrasound elastography is required for US assessment of muscle stiffness, but this type of measurements are nowadays implemented in many commercial US machines, however making them more expensive. The EI is an US parameter that always requires some external software to allow a quantitative measurement, otherwise remains as a subjective qualification made by the operator. Echo-intensity can be derived from the digital backscattered radiofrequency signal, but it is rarely viable in clinical context due to the limitations of US equipment and the need for custom signal processing (Zaidman et al., 2012).

1.4.2 Ultrasound image quantitative parameters

The quantitative estimation of echogenicity in clinical settings is often made through grayscale histogram analysis. This imaging analysis technique involves the construction of a plot featuring the number of pixels associated with a given ROI within intervals determined by intensity level (Pillen et al., 2009). Post-image acquisition analysis may be performed using a variety of image editing programs.

Many image analysis platforms are available and differ based on file type constraints, software customization and flexibility, hardware requirements, cost limitations, and image visualization needs (e.g., confocal microscopy, CT imaging, US imaging, etc.). Options range from commercially available software such as AnalyzeDirect, Inc., and SliceOmatic (TomoVision, Canada), to open source software options such as OpenCV, GNU Image Manipulation Program, Medical Imaging Interaction Toolkit, MIPAV (Medical Image Processing, Analysis, and Visualization), and OsiriX (Harris-Love et al., 2014). Software as Matlab (MATLAB and Image Processing Toolbox 2014a, MathWorks) requires some script writing skill, which combined with the cost of this software, limits its use. Nevertheless, the open nature of Matlab package allows custom usage and further advancement of the quantification methods (Smith & Barton, 2014).

To overcome the subjective nature of some of the US measures, there are many software packages available on the market. A commonly cited program for grayscale histogram analysis is Photoshop (Adobe Systems, San Jose, CA), which has been generally used for clinical applications ranging from the quantitative analysis of endothelial damage to the measurement of skeletal muscle echogenicity in older adults (Harris-Love et al., 2014).

Another alternative for image analysis is Image J (National Institutes of Health, Bethesda, MD, version 1.45s), a public-domain Java-based image processing and analysis program developed by Wayne Rasband of the National Institute of Mental Health at the National Institutes of Health (NIH). Image J has been extensively used for image processing in immunohistochemistry, tissue segmentation in microscopy images, and muscle morphometric measurements (Fortin & Battie, 2012; Schneider et al., 2012). This software continues to push and drive the field by sticking to a core set of design principles that have allowed it to become a modern image-processing platform (Schneider et al., 2012). Image J's third-party tool connections have allowed it to be used in image workflows and take advantage of algorithm capabilities provided by Matlab. This package advocates a more flexible approach that would allow users to add new functionalities that are then shared with others. This was accomplished through the use of macros (custom programming scripts that automate tasks inside a large piece of software) and plugins. Image J has since evolved in its scripting capabilities and now allows other scripting environments to be harnessed, such as JavaScript, or other languages to be called, such as Python, through an Image J Jython Bridge (Schneider et al., 2012). Photoshop and Image J have both been cited as being among the most frequently used image processing and analysis software (Nanes, 2015).

Both Photoshop and Image J have a variety of selection tools for drawing ROIs within an image. Two commonly used methods include the semi-automated creation of a square or rectangular ROI region, or tracing the ROI region using a series of line segments that closely align with the targeted anatomical or morphological structure (Harris-Love et al., 2014).

The 32-bit OsiriX software (version 3.8.1, Pixmeo, Geneva, Switzerland) was previously assessed as a more user-friendly image analysis software package for the Apple Mac OS (Microsoft Corp, Redmond, Washington) than Image J. One of the OsiriX software's main advantages is its integrated picture archiving and communication system, which allows patient data to be stored automatically. Both OsiriX and Image J packages are used by clinicians and investigators in a wide variety of studies as functional tools for image analysis and they reveal excellent inter-software reliability and agreement (Fortin & Battie, 2012).

Image quality can be analysed in two modes: objective and subjective (Jaffe et al., 2006; Tang et al., 2012). Objective image analysis can be determined by measuring the mean pixel value and corresponding standard deviation (SD), these values facilitate the analyses of signal (as EI in US) and noise respectively (Söderberg et al., 2010). The inclusion of US image quality evaluation using both subjective and objective methods is important to strengthen the role of MSK ultrasound examination.

1.5 Muscle contraction exercise

Today there is a growing focus of people on pursuing a healthy and fit lifestyle, which has led to a great increase in the number of people who practice fitness and athletic activities. Demographic data suggest a large number of persons are engaged in regular exercise and structured sporting activities (Alqahtani, 2010). Such increased physical activity among the general population improves functional strength and benefits health, contributes to disease prevention and is an important adjunct in rehabilitation programs (Norrbrand, 2010).

Strength training or muscular endurance is important to improve health and fitness. The benefits of strength training include the development and maintenance of muscular strength and endurance, the prevention and rehabilitation of muscular injuries and the reduction of the risk for some chronic diseases. Muscular action or neuromuscular activation of muscles contributes to the movement or stabilization of the MSK system (Walker et al., 2004; Walker, 1968).

Muscular actions can be of three types: isometric, eccentric (Ecc), and concentric (Conc). The isometric action happens when a force of equal magnitude opposes the amount of force produced by a muscle so that the length of the whole muscle-tendon complex remains unchanged. In fact, true isometric contractions do not occur during natural contractions, since a small degree of muscle fascicles shortening always occur as a result of the stretching of the series elastic connective tissue. Therefore, MT increases during isometric contractions and there are visible changes in the position of the muscle fascicles, especially when viewed transversally (Walker et al., 2004; Walker, 1968).

During Conc and Ecc muscle contractions, the muscle shortens and lengthens, respectively. A Conc contraction occurs when the torque of the activated muscles is greater than the resistance torque, resulting in muscle shortening (Knudson, 2007; Wang, 2011). The Ecc action occurs when the muscle is forced to stretch because the force that generated is insufficient to overcome the external load, i.e., the torque of the activated muscles is less than the torque of the resistance (Knudson, 2007). Skeletal muscle possesses the inherent capability to produce greater force during Ecc contractions than during Conc ones (Knudson, 2007). However, the metabolic cost of Ecc contractions is lower, and thus mechanical efficiency is higher during Ecc contractions compared to Conc contractions. Because Ecc and Conc contractions potentially offer different stimulus dictating hypertrophy, neural drive and protein metabolism, it is generally agreed that resistance exercise should comprise both Conc and Ecc actions (Baptista et al., 2016). It should also be recalled that most daily activities and movements, e.g., walking, climbing, or lifting objects, are carried out using coupled Conc and Ecc muscle contractions (Norrbrand, 2010).

When the intensity of muscle contractions, especially of Ecc contractions, reaches levels higher than those the individual is accustomed, active muscles suffer a number of alterations that often result in pain (delayed onset muscle soreness), which is accompanied by oedema and increased stiffness that last from several hours to days after exercise (Knudson, 2007).

In addition to the type of muscular action, it is known that the duration and intensity, associated to the number of repetitions, sets, rest time and training frequency lead to visible morphological changes in the trained muscles (Cadore et al., 2012; Knudson, 2007). These changes may be more or less perceptible and their evaluation is extremely important.

Strength training and/or regular exercise can alter the muscle appearance on US images by varying the EI of the muscle. Muscles submitted to exercise training generally have lower EI due to the volume effect caused by the increase in myocyte size relative to the perimisial tissue volume, as well as the increase in vascularization (Alqahtani, 2010; Trip et al., 2009; Vlychou & Teh, 2008; Walker et al., 2004).

Eccentric muscle strength training seems to be very efficacious in preventing and recovering from MSK system injuries, in increasing muscle strength and in improving the mechanical resistance of muscles' connective tissue, and in promoting neural adaptations (Cadore, 2012). This type of training is commonly used for faster rehabilitation and to increase muscle performance in athletes and non-athletes (Santos et al., 2014; Silva et al., 2011). The importance of Ecc muscle exercise seems to be related to induced muscle adaptations caused by the heightened mechanical loading and muscle damaged caused by Ecc muscle contractions. This type of contraction causes more muscle damage than other types of muscular activity and when few Ecc muscle contractions are performed, this has a protective effect on the muscle. On the other hand, it has been reported that Conc strength training does not reduce or prevent muscle damage and may produce a negative effect on Ecc exercise-induced muscle damage (Silva et al., 2011). Eccentric training induces lower Conc but greater Ecc strength increases, compared with Conc training (Cadore et al., 2014).

Recently, Ecc training-induced adaptations have been widely examined due to their positive effects on strength performance, muscle hypertrophy, and injury rehabilitation in athletes as well as in healthy untrained subjects. Eccentric muscle contractions involve unique neuromuscular features that are partly distinct from Conc muscle actions. Some of these differences are greater muscle force production, lower metabolic cost, lower neuromuscular activity required to produce the same workload, lower neuromuscular activity prior to the onset of movement, and greater muscle damage. Thus, Ecc and Conc actions provide different stimuli to the muscles and therefore may induce different neural and morphological adaptations (Cadore et al., 2014).

Muscle quality can be assessed using US imaging. Enhanced EI is thought to represent changes caused by increases in intramuscular connective and adipose tissues (Cadore et al., 2012; Pillen et al., 2009). In addition, traditional resistance training may improve muscle quality, as suggested by a reduced EI observed after 6 weeks of training in elderly adults (Cadore et al., 2014; Radaelli et al., 2012).

Although the prolonged performance of resistance training provides many neuromuscular benefits, resistance training is usually performed to develop the strength and size of muscle. However, the underlying physiological mechanisms linking the configuration of the resistance-training program to the chronic adaptations has yet to be clearly defined (Branderburg & Docherty, 2006).

Chronic resistance exercise sometimes referred to as strength training or weight training, promotes increases in muscle strength, power and size, and is employed to enhance athletic performance. Typically, resistance exercise is performed with coupled shortening (Conc) and lengthening (Ecc) muscle actions, while the number of sets and repetitions vary depending on the specific aim of the training. Thus, resistance exercises for athletes vary from ballistic exercises to improve explosive strength for e.g. track and field athletes, to the extremely heavy loading often used by power lifters. Resistance exercise has been practiced for centuries, and despite several studies on this theme, controversy still prevails regarding training load, number of sets and repetitions, rest periods in order to achieve an optimized training response (Norrbrand, 2010).

1.6 Anatomical features of the quadriceps femoris

The extensor mechanism of the knee joint has been extensively studied. This mechanism consists of a complex arrangement of various muscles, tendons, ligaments, and soft-tissue structures, which include the quadriceps femoris muscle (Andrikoula et al., 2006). The quadriceps femoris muscle consists of four distinct muscle heads that share a common insertion tendon, the quadriceps tendon. This tendon is trilaminar, with the anterior layer formed by the RF, the intermediate layer formed by the VM and VL and the deep layer by the tendon of the VI (Figure 8) (Andrikoula et al., 2006).

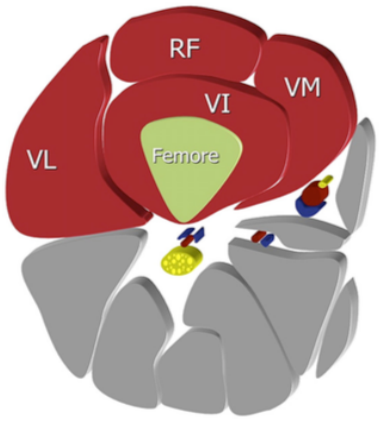


Figure 8: Diagram showing the anatomy of the quadriceps femoris in axial plane. VL, vastus lateralis; VI, vastus intermedius; VM, vastus medialis; RF, rectus femoris (Pasta et al., 2010).

The RF (Figure 9) is a long fusiform muscle and is separated from the other muscles along its entire course except at the patellar insertion. Proximally, the RF is unipennate, however distally it is blended to the form a u-shape, in which the superficial fibres are bipennate and the deep ones are parallel. The fibre lengths are similar throughout the entire RF (Farahmand et al., 1998). At the distal end, the RF narrows and continues by a tendon that is inserted into the superior pole of the patella (Andrikoula et al., 2006).

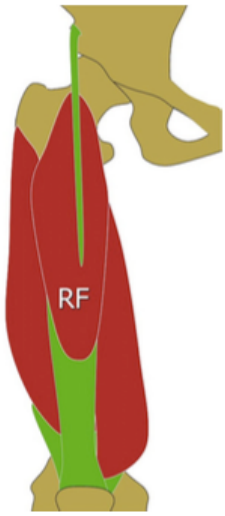


Figure 9: Anatomy of the quadriceps femoris in a superficial plane. RF, rectus femoris (Pasta et al., 2010).

The VL is generally the largest component of the quadriceps femoris. This muscle head has a unipennate architecture with its fibres originating from a broad superficial aponeurosis that covers the muscle proximally. These fibres then course distally and medially and join an aponeurosis on the deep aspect of the thigh distally that then tapers to form a flat tendon that attaches to the superior and superolateral borders of the patella. The vastus lateralis obliquus (VLO) can be distinguished from the main size of the VL by an abrupt change of fibre alignment (Farahmand et al., 1998). The VL extends halfway down, giving off a fibrous expansion that blends with the lateral patellar retinaculum, through which the VL attaches directly to the tibia (Andrikoula et al., 2006).

The VM muscle head is unipennate and its muscle fibres take a helical course from their origin to insertion (Farahmand et al., 1998). The attachments of the VM's muscle fascicles form most of the intermediate layer of the quadriceps tendon. However, the

most distal muscle fibres of the VM course almost horizontally and anteriorly towards their insertion into the common tendon and the medial border of the patella. This part of the muscle is sometimes described as the vastus medialis obliquus (VMO). Like the VL, the VM possesses a distal fibrous expansion that mingles into the medial patellar retinaculum. The VMO muscle is reported to be the primary stabilizer of the patella during knee extension (Andrikoula et al., 2006).

The VI is also unipennate with an extensive femoral origin. The VI's muscle fibres end in an anterior aponeurosis that forms the deepest layer of the quadriceps tendon (Farahmand et al., 1998). The VI partly blends with the VM medially (Andrikoula et al., 2006).

In conclusion, the muscle fibres of the RF and VI insert both into the superior pole of the patella and are almost aligned with the proximal-distal direction, whereas the fibres of the VM and VL join the patella obliquely (Andrikoula et al., 2006) (Figure 10).

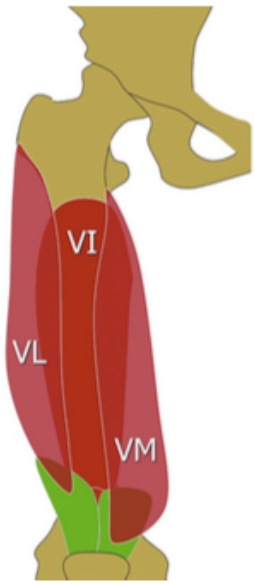


Figure 10: Anatomy of the quadriceps muscle in a deep plane. VL, vastus lateralis; VI, vastus intermedius; VM, vastus medialis (Pasta et al., 2010).

The four heads of the quadriceps converge distally to the tendon of the quadriceps, which is inserted in the upper edge of the patella (Pasta et al., 2010; Waligora et al., 2009). The tendon is composed by branches that are positioned on top of each other. The superficial branch is continuous to the muscle fibres of the RF. The intermediate branch receives the fibres of the VM and VL, and the deepest branch receives the fibre of the VI (Pasta et al., 2010; Standring, 2008). A small quantity of fibres of the

superficial branch merges directly into the patellar tendon (Waligora et al., 2009). The description given is generally accepted, although recent studies based on anatomic dissections have revealed a considerable variability in the composition of the distal tendon of the quadriceps femoris (Pasta et al., 2010; Waligora et al., 2009).

The forces generated by the quadriceps femoris are crucial for the knee joint biomechanics, due to their role in controlling tibiofemoral and patellofemoral kinematics, and cartilage contact forces, and the stresses applied to the knee joint capsule and ligaments. However, most of the time it is difficult to obtain direct, *in vivo*, measurements of these loads. The quadriceps femoris' heads possess musculotendinous junctions that join quadriceps tendons from different angles, such that the tendon line-of-action (defined as the unit vector from tendon insertion on the patella to its muscular origin) varies across the width of the tendon. Therefore, six lines-of-action were used to characterize the action of the whole quadriceps femoris. The central quadriceps components, VI and RF, were each characterized by a single line-of-action representing the central muscle fibres. The VI and RF origins were chosen as the centre point of each musculotendinous junction and both lines-of-action inserted on the patellar apex, defined as the midpoint of the most proximal edge of the patella. For the VM and VL two lines-of-action were defined for each musculotendinous unit: a proximal portion (VM proximal and VL proximal) and the distal portion (VM distal and VL distal) (Wilson & Sheehan, 2011).

The quadriceps force vector (Figure 11) includes forces that are generated by contraction of the VL, VI, RF, VM. The VL is composed by two force vector components, the vastus lateralis longus (VLL) and VLO. The VM is composed of two force vector components, the VM longus (VML) and VMO. In the coronal plane, the quadriceps force vector angles are made by the VLO at 35º and the VLL at 14º laterally, by the VI and RF at 0º, and medially by VMO at 47º and VML at 15º. Overall, the quadriceps force has a posterior pull to keep the patella in proper articulation with the trochlear groove. In normal patients, no timing difference between the contraction of the VMO and VL exists. VMO training is important to improve VL and VMO onset timing differences (Waryasz & McDermott, 2008).

Various studies have described the possible role of anatomic variations in the quadriceps mechanism in relation to abnormal conditions (Andrikoula et al., 2006). Quadriceps femoris muscle strengthening is useful for improving functional ability and may be particularly important for individuals who want to return to higher-demand activities, such as running or other sports. Previous studies have emphasized the importance of quadriceps strengthening in patients with patellofemoral pain (Eapen et al., 2011).

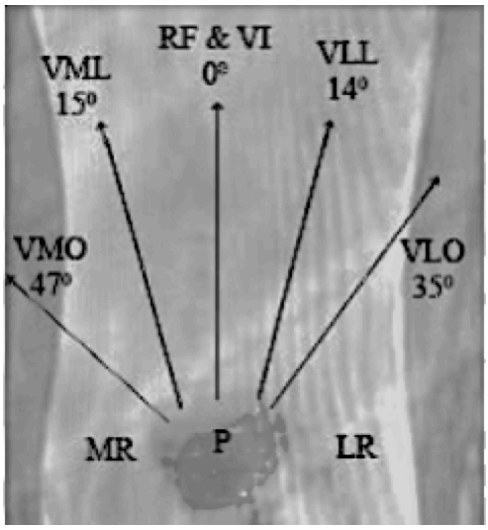


Figure 11: Quadriceps patellar force diagram. MR, medial retilaculum; P, patella; LR, lateral retilaculum; VLO, vastus lateralis obliquus; VLL, vastus lateralis longus; RF, rectus femoris; VI, vastus intermedius; VML, vastus medialis longus; VMO, vastus medialis obliquus (Waryasz et al., 2008).

Eccentric training of quadriceps femoris muscle is effective in reducing pain and improving the functional status of patients with patellofemoral pain syndrome and can be suggested as part of treatment. Quadriceps femoris muscle strengthening is useful for improving functional ability, in athletes, aging people or in some knee pathologies (Andrikoula et al., 2006; Eapen et al., 2011; Farahmand et al., 1998; Waryasz & McDermott, 2008).

1.7 Summary

Ultrasound is an important image modality for MSK evaluation and to assess muscle adaptations to a variety of stimuli (Nishihara et al., 2014; Rech et al., 2014). Advantages of US imaging include non-invasiveness, low cost, absence of ionising radiation, and the possibility to perform dynamic assessments (Lento & Primack, 2008). The study of the skeletal muscle adaptations to strength training by means of US

imaging has grown at a very fast pace in the last years. Important reasons for this growth includes the use of elastography, a non-invasive technique that allows the characterisation of the mechanical properties of tissues (Gennisson et al., 2013) and the development of methods for quantifying changes in EI.

The quadriceps muscle is one of the crucial factors for the knee joint biomechanics, because it contributes for the control of tibiofemoral kinematics, patellofemoral kinematics, and cartilage contact forces. The morphology and function of the quadriceps femoris are very complex and the way its various components contribute to the whole function of the knee extensor mechanism and how they adapt to different kinds of strength training are still open questions. Due to their capabilities, quantitative US imaging might be helpful in answering these questions.

1.8 References

Abella, B., & Zordo, T. (2008). Elastography breaks new ground in musculoskeletal imaging. *Diagnostic Imaging*, *December 1*, 1–2.

Alqahtani, M. (2010). *Quantitative Ultrasound Image Analysis of the Gastrocnemius Muscle for Injury Evaluation*. Cardiff University.

Andonian, P., Viallon, M., Le Goff, C., Bourguignon, C., Tourel, C., Morel, J., … Croisille, P. (2016). Shear-wave elastography assessments of quadriceps stiffness changes prior to, during and after prolonged exercise: A longitudinal study during an extreme mountain ultra-marathon. *PLoS ONE*, *11*(8).

Andrikoula, S., Tokis, A., Vasiliadis, H., & Georgoulis, A. (2006). The extensor mechanism of the knee joint: An anatomical study. *Knee Surgery, Sports Traumatology, Arthroscopy*, *14*(3), 214–220.

Baptista, R., Onzi, E., Goulart, N., Dos Santos, L., Makarewicz, G., & Vaz, M. (2016). Effects of Concentric Versus Eccentric Strength Training on the Elderly ' s Knee Extensor Structure and Function. *Journal of Exercise Physiology Online*, *19*(3), 120–133.

Barr, R., Nakashima, K., Amy, D., Cosgrove, D., Farrokh, A., Schafer, F., … Kudo, M. (2015). WFUMB Guidelines and Recommendations for Clinical Use of Ultrasound Elastography: Part 2; Breast. *Ultrasound in Medicine & Biology*, *41*(5), 1148–1160.

Blankenbaker, D., & Smet, A. (2006). The role of ultrasound in the evaluation of sports injuries of the lower extremities. *Clinics in Sports Medicine*, *25*(4), 867–97.

Boykin, R., Heuer, H. Vaishnav, S., Millett, P. (2010). Rotator cuff disease – basics of diagnosis and treatment. Rheumatology Reports, 2(1).

Bouillard, K., Jubeau, M., Nordez, A., & Hug, F. (2014). Effect of vastus lateralis fatigue on load sharing between quadriceps femoris muscles during isometric knee extensions. *Journal of Neurophysiology*, *111*(4), 768–776.

Bouillard, K., Nordez, A., & Hug, F. (2011). Estimation of individual muscle force using elastography. *PloS One*, *6*(12), e29261.

Brandenburg, J., Eby, S., Song, P., Zhao, H., Brault, J., Chen, S., & An, K. (2014). Ultrasound elastography: The new frontier in direct measurement of muscle stiffness. *Archives of Physical Medicine and Rehabilitation*, *95*(11), 2207–2219.

Branderburg, J., & Docherty, D. (2006). The effect of training volume on the acute response and adaptation to resistance training. *Int. J Sports Physiol. and Performance*, *1*, 108–121.

Cadore, E. (2012). *Strength and endurance training in elderly men. (Tese de Doutoramento)*. Public University of Navarre.

Cadore, E., Izquierdo, M., Conceição, M., Radaelli, R., Pinto, R., Baroni, B., … Kruel, L. (2012). Echo intensity is associated with skeletal muscle power and cardiovascular performance in elderly men. *Experimental Gerontology*, *47*(6), 473–8.

Cadore, E., González-Izal, M., Pallarés, J., Rodriguez-Falces, J., Häkkinen, K., Kraemer, W., … Izquierdo, M. (2014). Muscle conduction velocity, strength, neural activity, and morphological changes after eccentric and concentric training. *Scandinavian Journal of Medicine and Science in Sports*, *24*(5), 343–352.

Caresio, C., Molinari, F., Emanuel, G., & Minetto, M. (2015). Muscle echo intensity: Reliability and conditioning factors. *Clinical Physiology and Functional Imaging*, *September*(5), 1–11.

Castaneda, B., Rubens, D., & Parker, K. (2010). Clinical applications of sonoelastography. *2010 Pan American Health Care Exchanges*, 74–75.

Cavalcanti, E. (2012). *Desenvolvimento de um Sistema para Medida Elastográfica Dinâmica por Ultrassom*. Universidade de São Paulo.

Chino, K., Akagi, R., Dohi, M., Fukashiro, S., & Takahashi, H. (2012). Reliability and Validity of Quantifying Absolute Muscle Hardness Using Ultrasound Elastography, *7*(9), 5–9.

Cosgrove, D., Piscaglia, F., Bamber, J., Bojunga, J., Correas, J., Gilja, O., … Dietrich, C. (2013). EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology. *Ultraschall Medicine*, *34*(3), 238–53.

Cosgrove, D., Piscaglia, F., Bamber, J., Bojunga, J., Gilja, O., Klauser, A., … Words, K. (2013). EFSUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography. Part 2 : Clinical Applications. *Ultraschall in Medicine*, *34*(3), 238–253.

D'Onofrio, M., Crosara, S., Robertis, R., Canestrini, S., Demozzi, E., & Pozzi Mucelli, R. (2014). Elastography of the pancreas. *European Journal of Radiology*, *83*(3), 415–9.

Delaney, S., Worsley, P., Warner, M., Taylor, M., & Stokes, M. (2010). Assessing contractile ability of the quadriceps muscle using ultrasound imaging. *Muscle & Nerve*, *42*(4), 530–8.

Drakonaki, E., Allen, G., & Wilson, D. (2012). Ultrasound elastography for musculoskeletal applications. *The British Journal of Radiology*, *85*(1019), 1435–45.

Eapen, C., Nayak, C., & Zulfeequer, C. (2011). Effect of Eccentric Isotonic Quadriceps Muscle Exercises on Patellofemoral Pain Syndrome: An Exploratory Pilot Study. *Asian Journal of Sport Medicine*, *2*(4), 227–234.

Farahmand, F., Senavongse, W., & Amis, A. (1998). Quantitative Study of the Quadriceps Muscles and Trochlear Groove Geometry Related to Instability of the Patellofemoral Joint. *J Orthop Res.*, *16*(1), 136–43.

Fierbinteanu-Braticevici, C., Usvat, R., Oprea, G., Sarbu, E., Petrisor, A., & Panaitescu, E. (2012). Role of Acoustic Radiation Force Impulse (Arfi) Sonoelastography for Noninvasive Diagnosis of Nonalcoholic Fatty Liver Disease. *Journal of Hepatology*, *56*, S413.

Fortin, M., & Battie, M. (2012). Quantitative Paraspinal Muscle Measurements: Inter-Software Reliability and Agreement Using OsiriX and ImageJ. *Physical Therapy*, *92*(6), 853–864.

Franchi-Abella, S., Elie, C., & Correas, J. (2013). Ultrasound elastography: advantages, limitations and artefacts of the different techniques from a study on a phantom. *Diagnostic and Interventional Imaging*, *94*(5), 497–501.

Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., … Ichihashi, N. (2012). Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *European Journal of Applied Physiology*, *112*(4), 1519–25.

Fulton, R. (2014). Focused — Basic Ultrasound Principles and Artifacts. In *Focused Ultrasound Techniques for the Small Animal Pratitioner* (1st ed., pp. 1–16).

Gennisson, J., Deffieux, T., Fink, M., & Tanter, M. (2013). Ultrasound elastography: principles and techniques. *Diagnostic and Interventional Imaging*, *94*(5), 487–95.

Gennisson, J., Deffieux, T., Macé, 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 and Biology*, *36*(5), 789–801.

Gheorghe, L., Iacob, S., Iacob, R., Dumbrava, M., Becheanu, G., Herlea, V., … Popescu, I. (2009). Real time elastography - a non-invasive diagnostic method of small hepatocellular carcinoma in cirrhosis. *Journal of Gastrointestinal and Liver Diseases*, *18*(4), 439–446.

Goddi, A., Sacchi, A., Magistretti, G., & Almolla, J. (2011). Transrectal real-time elastography of the prostate: Normal patterns. *Journal of Ultrasound*, *14*(4), 220–232.

Håkansson, M., Svensson, S., Zachrisson, S., Svalkvist, A., Båth, M., & Månsson, L. (2010). ViewDEX: An efficient and easy-to-use software for observer performance studies. *Radiation Protection Dosimetry*, *139*(1–3), 42–51.

Hammond, K., Mampilly, J., Laghi, F., Goyal, A., Collins, E., McBurney, C., … Tobin, M. (2014). Validity and reliability of rectus femoris ultrasound measurements: Comparison of curved-array and linear-array transducers. *Journal of Rehabilitation Research and Development*, *51*(7), 1155–1164.

Harris-Love, M., Monfaredi, R., Ismail, C., Blackman, M., & Cleary, K. (2014). Quantitative

ultrasound: Measurement considerations for the assessment of muscular dystrophy and sarcopenia. *Frontiers in Aging Neuroscience*, *6*(JUL), 1–4.

Hirono, J., Mukai, N., Takayanagi, S., & Miyakawa, S. (2016). Changes in the hardness of the gastrocnemius muscle during a Kendo training camp as determined using ultrasound real-time tissue elastography. *The Journal of Physical Fitness and Sports Medicine*, *5*(3), 239–245.

Iglesias-Garcia, J., Larino-Noia, J., Abdulkader, I., Forteza, J., & Dominguez-Munoz, J. (2009). EUS elastography for the characterization of solid pancreatic masses. *Gastrointestinal Endoscopy*, *70*(6), 1101–1108.

Jaffe, T., Nelson, R., Johnson, G., Lee, E., Yoshizumi, T., Lowry, C., … Paulson, E. (2006). Optimization of multiplanar reformations from isotropic data sets acquired with 16-detector row helical CT scanner. *Radiology*, *238*(1), 292–9.

Jansen, M., Alfen, N., Sanden, M., Dijk, J., Pillen, S., & Groot, I. (2012). Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. *Neuromuscular Disorders*, *22*(4), 306–317.

Kim, S., Park, H., & Lee, S. (2015). Usefulness of strain elastography of the musculoskeletal system. *Ultrasonography*, *35*(April), 104–109.

Kinugasa, R., Yoshida, K., Watanabe, T., Kuchiki, K., & Horii, A. (2005). Skin cooling alters the activation patterns of different heads of the quadriceps. *Canadian Journal of Applied Physiology*, *30*(2), 127–139.

Klauser, A., Miyamoto, H., Bellmann-Weiler, R., Feuchtner, G., Wick, M., & Jaschke, W. (2014). Sonoelastography: musculoskeletal applications. *Radiology*, *272*(3), 622–33.

Knudson, D. (2007). *Fundamentals of Biomechanics* (Second). USA: Springer.

Konofagou, E., Ophir, J., & Krouskop, T. (2003). Elastography: From Theory To Clinical Applications. In *Summer Bioengineering Conference* (Vol. June, pp. 367–368). Florida.

Kudo, M., Shiina, T., Moriyasu, F., Iijima, H., Tateishi, R., Yada, N., … Kumada, T. (2013). JSUM ultrasound elastography practice guidelines: liver. *Journal of Medical Ultrasonics*, *40*(4), 325–357.

Lacourpaille, L., Hug, F., Bouillard, K., Hogrel, J., & Nordez, A. (2012). Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. *Physiological Measurement*, *33*(3), N19-28.

Ledenius, K., Svensson, E., Stålhammar, F., Wiklund, L., & Thilander-Klang, A. (2010). A method to analyse observer disagreement in visual grading studies: Example of assessed image quality in paediatric cerebral multidetector CT images. *British Journal of Radiology*, *83*(991), 604–611.

Lee, J., & Healy, J. (2004). Sonography of lower limb muscle injury. *American Journal of Roentgenology*, *182*(2), 341–51.

Lee, R. (2009). Ultrasound elastography accurately spots skin cancer. *Diagnostic Imaging*, *December*(3), 1–2.

Lento, P., & Primack, S. (2008). Advances and utility of diagnostic ultrasound in musculoskeletal medicine. *Current Reviews in Musculoskeletal Medicine*, *1*(1), 24–31.

McNally, E. (2011). The development and clinical applications of musculoskeletal ultrasound. *Skeletal Radiology*, *40*(9), 1223–1231.

Monetti, G., & Minafra, P. (2007). The musculoskeletal elastography. *MEDIX Suppl.*, 43–45.

Muraki, S., Fukumoto, K., & Fukuda, O. (2013). Prediction of the muscle strength by the muscle thickness and hardness using ultrasound muscle hardness meter. *SpringerPlus*, *2*(457), 1–7.

Nanes, B. (2015). *Slide Set : Reproducible image analysis and batch processing with ImageJ*. *BioTecniques* (Vol. 59).

Neves, L. (2002). *Otimização do algoritmo de block matching aplicado a estudos elastográficos*.

São Paulo. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.

Nielsen, P., Jensen, B., Darvann, T., Jørgensen, K., & Bakke, M. (2006). Quantitative ultrasound tissue characterization in shoulder and thigh muscles--a new approach. *BMC Musculoskeletal Disorders*, *7*, 2.

Nishihara, K., Kawai, H., Hayashi, H., Naruse, H., Kimura, A., Gomi, T., & Hoshi, F. (2014). Frequency analysis of ultrasonic echo intensities of the skeletal muscle in elderly and young individuals. *Clinical Interventions in Aging*, *9*, 1471–1478.

Nordez, A., Cornu, C., & McNair, P. (2006). Acute effects of static stretching on passive stiffness of the hamstring muscles calculated using different mathematical models. *Clinical Biomechanics*, *21*(7), 755–760.

Norrbrand, L. (2010). *Acute and Early Chronic Responses To Resistance Exercise Using Flywheel or Weights*. Mid Sweden University.

Ophir, J. (2005). A Review of Theoretical and Experimental Aspects of Imaging the Elastic Attributes of Tissue In Vivo. In *Research and Development in Breast Ultrasound* (pp. 5–8). Springer.

Ophir, J., Kallel, F., Varghese, T., Bertrand, M., Cespedes, I., & Ponnekanti, H. (1997). Elastography : A Systems Approach. *John Wiley & Sons, Inc*, *8*(1), 89–103.

Paluch, Ł., Nawrocka-Laskus, E., Wieczorek, J., Mruk, B., Frel, M., & Walecki, J. (2016). Use of Ultrasound Elastography in the Assessment of the Musculoskeletal System. *Polish Journal of Radiology*, *81*, 240–246.

Papadakis A., Perisinakis K., & Damilakis J. (2008). Automatic exposure control in pediatric and adult multidetector CT examinations: A phantom study on dose reduction and image quality. *Medical Physics*, *35*(10), 4567.

Pasta, G., Nanni, G., Molini, L., & Bianchi, S. (2010). Sonography of the quadriceps muscle: Examination technique, normal anatomy, and traumatic lesions. *Journal of Ultrasound*, *13*(2), 76–84.

Pedersen, M., Fredberg, U., Langberg, H., Hospital, B., Silkeborg, R., Rehab, C., … Sciences, H. (2012). Sonoelastography as a Diagnostic Tool in the Assessment of Musculoskeletal Alterations : A Systematic Review Sonoelastografie als Diagnoseverfahren, um muskuloskelettale Veränderungen. *Ultraschall in Medicine*, *33*, 441–446.

Petscavage-Thomas, J. (2014). Clinical applications of dynamic functional musculoskeletal ultrasound. *Reports in Medical Imaging*, *7*(1), 27–39.

Pillen, S. (2010). Skeletal muscle ultrasound. *European Journal Translational Myology*, *1*(4), 145–155.

Pillen, S., Arts, I., & Zwarts, M. (2008). Muscle ultrasound in neuromuscular disorders. *Muscle & Nerve*, *37*(6), 679–693.

Pillen, S., Dijk, J., Weijers, G., Raijmann, W., Korte, C., & Zwarts, M. (2009). Quantitative gray-scale analysis in skeletal muscle ultrasound: a comparison study of two ultrasound devices. *Muscle & Nerve*, *39*(June), 781–786.

Pillen, S., Keimpema, M., Nievelstein, R., Verrips, A., Kruijsbergen-Raijmann, W., & Zwarts, M. (2006). Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound in Medicine and Biology*, *32*(9), 1315–1321.

Pillen, S., Tak, R., Zwarts, M., Lammens, M., Verrijp, K., Arts, I., … Verrips, A. (2009). Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound in Medicine & Biology*, *35*(3), 443–6.

Pochini, A., Ferretti, M., Kawakami, E., Fernandes, A., Yamada, A., Oliveira, G., … Ejnisman, B. (2015). Analisys of pectoralis major tendon in weightlifting athletes using ultrasonography and elastography. *Einstein (São Paulo)*, *13*(4), 541–546.

Radaelli, R., Bottaro, M., Wilhelm, E., Wagner, D., & Pinto, R. (2012). Time Course of Strength

and Echo Intensity Recovery After Resistance Exercise in Women. *Journal of Strength and Conditioning Research*, *26*(9), 2577–2584.

Radaelli, R., Neto, E., Marques, M., & Pinto, R. (2011). Espessura e qualidade musculares medidas a partir de ultrassonografia: Influ??ncia de diferentes locais de mensura????o. *Revista Brasileira de Cineantropometria E Desempenho Humano*, *13*, 87–93.

RCR. (2012). *iRefer- Making the best use of clinical radiology- The Royal College of Radiologists* (seventh-20). London.

Rech, A., Radaelli, R., Goltz, F., Rosa, L., Schneider, C., & Pinto, R. (2014). Echo intensity is negatively associated with functional capacity in older women. *Age*, *36*(5).

Remedios, D., Hierath, M., Ashford, N., Cavanagh, P., Grenier, P., Lloyd, C., … Vilgrain, V. (2014). European survey on imaging referral guidelines. *Insights Imaging*, *5*, 15–23.

Ríos-Díaz, J., Ferrando, A., Martínez-Payá, J., & Aledo, M. (2010). Reliability and reproducibility of a morpho-textural image analysis method over a patellar ligament ultrasonography. *Reumatología Clinica*, *6*(6), 278–284.

Rizzatto, G. (2008). Ultrasound elastography. *Breast Cancer Research*, *10*(Suppl 3), P4.

Rumack, C., Wilson, S., & Charboneau, J. (2014). *Tratado de ultrassonografia diagnóstica (volume I)* (2ª). Guanabara-Koogan.

Salh, A. (2015). *Characterization of Shoulder Joint Morphology by Ultrasound*. National Ribat University.

Santos, H., Hanashiro, D., Ávila, M., Camargo, P., Oliveira, A., & Salvini, T. (2014). Efeito do treino isocinético excêntrico sobre a razão I/Q do torque e EMGs em sujeitos saudáveis. *Revista Brasileira de Medicina E Esporte*, *20*(3), 227–232.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to Image J : 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Silva, J., Job, L., Lanferdini, F., Rocha, C., & Vaz, M. (2011). Influência de 12 semanas de treinamento excêntrico nas razões de torque concêntrico e excêntrico de flexores/extensores do joelho. *Revista Brasileira de Engenharia Biomedica*, *27*(1), 39–45.

Smajlovic, F., Carovac, A., & Bulja, D. (2011). Sonoelastography : the method of choice for evaluation of tissue elasticity. *Journal of Health Sciences*, *1*(1), 50–55.

Smith, L., & Barton, E. (2014). SMASH – semi-automatic muscle analysis using segmentation of histology : a MATLAB. *Skeletal Muscle*, *4*(21).

Söderberg, M., Gunnarsson, M., & Nilsson, M. (2010). Simulated dose reduction by adding artificial noise to measured raw data: a validation study. *Radiation Protection Dosimetry*, *139*(1–3), 71–7.

Standring, S. (2008). *Gray's anatomy: The Anatomical Basis of Clinical Practice* (49th ed.). Churchill Livingstore.

Strasser, E., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–88.

Tan, A., Wakefield, R., Conaghan, P., Emery, P., & McGonagle, D. (2003). Imaging of the musculoskeletal system: Magnetic resonance imaging, ultrasonography and computed tomography. *Best Practice and Research: Clinical Rheumatology*, *17*(3), 513–528.

Tang, K., Wang, L., Li, R., Lin, J., Zheng, X., & Cao, G. (2012). Effect of low tube voltage on image quality, radiation dose, and low-contrast detectability at abdominal multidetector CT: phantom study. *Journal of Biomedicine & Biotechnology*, *2012*(Lcd), 130169.

Teixeira, A. (2013). *Efeito do treinamento de força na força, espessura muscular e qualidade muscular dos extensores do joelho de homens idosos*. Universidade Federal do Rio Grande do Sul.

Tilp, M., Steib, S., & Herzog, W. (2012). Length changes of human tibialis anterior central

aponeurosis during passive movements and isometric, concentric, and eccentric contractions. *European Journal of Applied Physiology*, *112*(4), 1485–94.

Toledo, M. (2016). *Reliability of ultrasound imaging measures of soft tissue stiffness using elastography in the posterior aspect of the leg*. Unitec.

Toursel, T., Stevens, L., Granzier, H., & Mounier, Y. (2002). Passive tension of rat skeletal soleus muscle fibers: effects of unloading conditions. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *92*(4), 1465–1472.

Trip, J., Pillen, S., Faber, C., Van Engelen, B., Zwarts, M., & Drost, G. (2009). Muscle ultrasound measurements and functional muscle parameters in non-dystrophic myotonias suggest structural muscle changes. *Neuromuscular Disorders : NMD*, *19*(7), 462–467.

Vega, M. (2011). *Métodos e análise para segmentação de imagens ultrassonográficas da mama*. Universidade de São Paulo.

Verhulst, F., Leeuwesteijn, A., Louwerens, J., Geurts, A., Van Alfen, N., & Pillen, S. (2011). Quantitative ultrasound of lower leg and foot muscles: feasibility and reference values. *Foot and Ankle Surgery*, *17*(3), 145–9. https://doi.org/10.1016/j.fas.2010.04.002

Vlychou, M., & Teh, J. (2008). Ultrasound of muscle. *Current Problems in Diagnostic Radiology*, *37*(5), 219–230.

Waligora, A., Johanson, N., & Hirsch, B. (2009). Clinical anatomy of the quadriceps femoris and extensor apparatus of the knee. *Clinical Orthopaedics and Related Research*, *467*(12), 3297–3306.

Walker, F., Cartwright, M., Wiesler, E., & Caress, J. (2004). Ultrasound of nerve and muscle. *Clinical Neurophysiology*, *115*(3), 495–507.

Walker, M. (1968). Effect of training on the properties of isolated skeletal muscles. *Experientia*, *24*(4), 360.

Wang, C. (2011). *Development of a vibro-ultrasound method for muscle stiffness measurement in vivo*. The Hong Kong Polytechnic University.

Wang, Q., Huang, Q., Yeow, J., Pickering, M., & Saarakkala, S. (2017). Quantitative Analysis of Musculoskeletal Ultrasound : Techniques and Clinical Applications. *BioMed Research International*, *2017*, 2–4.

Waryasz, G., & McDermott, A. (2008). Patellofemoral pain syndrome (PFPS): a systematic review of anatomy and potential risk factors. *Dynamic Medicine*, *7*(1), 9.

Whittaker, J., & Stokes, M. (2011). Ultrasound imaging and muscle function. *The Journal of Orthopaedic and Sports Physical Therapy*, *41*(8), 572–580.

Wilhelm, E., Rech, A., Minozzo, F., Radaelli, R., Botton, C., & Pinto, R. (2014). Relationship between quadriceps femoris echo intensity, muscle power, and functional capacity of older men. *Age*, *4*(Fev 11), 1–6.

Wilson, N., & Sheehan, F. (2011). Dynamic In Vivo Quadriceps Lines-of-Action. *J Biomech.*, *43*(11), 2106–2113.

Xu, J., Hug, F., & Fu, S. (2015). Stiffness of individual quadriceps muscle assessed using ultrasound shear wave elastography during passive stretching. *Journal of Sport and Health Science*, (August), 7–11.

Yanagisawa, O., Sakuma, J., Kawakami, Y., Suzuki, K., & Fukubayashi, T. (2015). Effect of exercise-induced muscle damage on muscle hardness evaluated by ultrasound real-time tissue elastography. *SpringerPlus*, *4*(1), 308.

Zaidman, C.; Holland, M.; Hughes, M. (2012). Quantitative ultrasound of skeletal muscle: reliable measurements of calibrated muscle backscatter from different ultrasound systems. *Ultrasound in Medicine & Biology*, *38*(9), 1618–1625.

Zaidman, C., Connolly, A., Malkus, E., Florence, J., & Pestronk, A. (2010). Quantitative ultrasound using backscatter analysis in Duchenne and Becker muscular dystrophy. *Neuromuscular Disorders : NMD*, *20*(12), 805–809.

Zaidman, C., Holland, M., Anderson, C., & Pestronk, A. (2008). Calibrated quantitative ultrasound imaging of skeletal muscle using backscatter analysis. *Muscle & Nerve*, *38*(1), 893–8.

Zordo, T., Lill, S., Fink, C., Feuchtner, G., Jaschke, W., Bellmann-Weiler, R., & Klauser, A. (2009). Real-time sonoelastography of lateral epicondylitis: comparison of findings between patients and healthy volunteers. *AJR. American Journal of Roentgenology*, *193*(1), 180–5.

Chapter 2 : Muscle characterisation by ultrasound in B mode

The subjective nature of EI evaluation, highly dependent on the operator, calls for the need to develop new quantitative methods that would allow a more objective assessment of tissues' EI (Alqahtani, 2010; Fukumoto et al., 2012; Pillen, 2010).

The quantitative analysis of the EI has recently been shown to be very useful for the diagnosis of neuromuscular diseases and also to study the muscle adaptations caused by physical exercise. Such quantitative analysis of the ultrasound images provides information that could not be detected by simple visual assessment (Alqahtani, 2010; Fukumoto et al., 2012).

In order to apply ultrasound imaging to the study of muscle adaptation, it is first necessary to study reproducibility of the measures gathered with this technique. Therefore, the general aim of this Chapter is to study the reproducibility of ultrasound measurements associated with muscle morphology and muscle tissue quality.

2.1 Reproducibility of ultrasound-derived muscle thickness and echo-intensity measures for the entire quadriceps femoris muscle

Imaging modalities are being increasingly employed to study skeletal muscles changes occurring due to disuse, ageing, training, or disease (Strasser et al., 2013). Compared to MRI, ultrasound is less expensive and more accessible (Vlychou & Teh, 2008). Furthermore, ultrasound equipment is portable and allows dynamic assessments to be performed in real time, which is useful in assessing physiological responses (Anderson & McDicken, 2002; Cohen et al., 1994; Pouch et al., 2010) and in diagnosing muscle injury and dysfunction (O'Sullivan et al., 2012; Zbojniewicz, 2014). Modern ultrasound technology has also greatly improved the quality of the ultrasound images and has widened the number of ultrasound imaging applications. The development of linear transducers with frequencies in the 7-15 MHz range has largely improved the scanning of more superficial structures and the visualization and delineation of the muscles and of their fascia and tendons, allowing fast and economical measurements of muscle architecture and composition to be made.

Muscle strength and function correlates with muscle mass and composition (Lafortuna et al., 2005). Changes in muscle mass happen relatively fast in response to strength training (Ahtiainen et al., 2003; Häkkinen et al., 1988), immobilization (Marimuthu et al., 2011), malnutrition (Norman et al., 2005), aging (Frontera et al., 2000; Roubenoff & Hughes, 2000), and disease (Andreassen et al., 2006; Bernard et al., 1998; Swallow et al., 2007). MT is a simple measure gathered from B-mode ultrasound images of muscles that is highly correlated with muscle cross sectional area. The reproducibility of ultrasound MT measurements is usually reported to be high or very high. This has been demonstrated for trunk (McMeeken et al., 2004; Wallwork et al., 2007; Wong et al., 2013), respiratory (Baldwin et al., 2011), and upper and lower limb muscles (Hammond et al., 2014; Lima et al., 2012), and for inter-session (Bunnell et al., 2015; Lima et al., 2012; Wallwork et al., 2007) and inter-rater measures (Baldwin et al., 2011; Hammond et al., 2014; Konig et al., 2014). Regarding muscle size assessment, the major disadvantage of ultrasound is that it only scans a rather limited area of the whole muscle. Also, slight changes in the orientation of the ultrasound probe might seriously affect MT measures. These drawbacks have hitherto been solved by standardizing the scanning region or by fixing the probe over the body segment, when this is feasible. Yet, MT measures' precision is similar in novices and experienced examiners (Wallwork

et al., 2007). In addition, the well-defined orientation of muscle fascicles aids in ultrasound probe placement when the muscle is scanned longitudinally.

Besides muscle mass, muscle composition also affects muscle function. More recently, muscle EI has been explored as a potential marker of muscle tissue status. The normal muscle appears in the ultrasound image (brightness mode) as a relatively hypoechoic structure, due to the rather low reflection of the ultrasound wave beam (low EI). In a transverse scan, muscles have a speckled appearance, which is explained by the higher EI of the perimysium surrounding muscle fiber bundles compared to that of the proper muscle tissue. The contrast in EI between muscle fascicles and the connective tissue of the perimysium is clearer in longitudinal scans and is very useful for further characterization of the muscle architecture, as well as for defining the muscle boundaries, taking advantage of the hyperechoic epimysium and overlying fascia (Blazevich et al., 2006; Konig et al., 2014; Strasser et al., 2013; Wilhelm et al., 2014).

The EI in an ultrasound scan can be measured simply as the average intensity of the pixels inside the muscle of interest, usually using a scale of levels of gray within a given ROI. Although a few studies confirm the good inter-session reliability of EI measures for muscles, there are still important questions about what would be the most desirable method for collecting such measures. One of the doubts regards ROI size that for some authors should include as much of the muscle as possible, avoiding bones and surrounding fascia. Imaging a whole section of the muscle would probably be important since internal fascia and non-homogenous distribution of EI might affect the measures. The orientation of the muscle bundles might also affect the reliability of EI measures, particularly in longitudinal scans (Caresio et al., 2014; Pasta et al., 2010; Wilhelm et al., 2014).

Some studies have investigated reliability of MT and EI using the quadriceps muscle (Blazevich et al., 2006; Wilhelm et al., 2014), although usually only for one of its four heads. However, the quadriceps femoris is anatomically and functionally complex and its different heads may adapt differently to training (Pasta et al., 2010). Due to their anatomy, different ultrasound examination techniques are required to image each of the four heads of the quadriceps femoris, thus potentially affecting the reliability of ultrasound measures (Pasta et al., 2010).

Therefore, this study assesses the intra and inter-session reliability (one week apart) of ultrasound measures of MT and EI in each of the four quadriceps femoris heads both in transverse and longitudinal scans and employing a rectangular ROI or the entire scanned section of the muscle.

2.1.1 Materials and Methods

Twenty healthy participants (10 females, mean ± standard deviation (SD); age=20.0±2.3 years; height=1.7±0.1 m; mass=64.2±10.9 kg; right thigh perimeter=52.0±3.8 cm; left thigh perimeter=51.7±4.1 cm) not engaged in sports or intense physical activities were informed about the study's protocol and procedures and gave written informed consent. Participants were excluded from the study if they sustained an injury in the lower extremity in the past six months, suffered from an orthopaedic condition or had surgery involving the lower extremities. Participants were also excluded if they have resistance trained their legs anytime during the past 12 months.

Procedures

To assess intra- and inter-session reliability of ultrasound measurements of MT and EI, three different ultrasound B-mode images were acquired bilaterally in transverse and longitudinal views from the four heads of the quadriceps muscle in two sessions, with an interval of 7 days between them. All participants were right-side dominant. To avoid possible effects related with daily routine, participants were evaluated at the same time of day in the two sessions and by the same examiner, a certified MSK ultrasound sonographer. After each scan, the transducer was moved away from the thigh and then placed back again over the same region of the thigh for the next scan.

Each head of the quadriceps muscle (VM, VL, RF and VI) was imaged with participants lying in supine, with the legs extended and relaxed. The ultrasound probe was placed over the midbelly region of each head of the quadriceps femoris, away from the patella at the following percentage of the distance between the upper edge of the patella and the superior iliac spine: 22% for VM, 39% for VL, and 56% for RF and VI (Blazevich et al., 2006).

A portable ultrasound machine (LOGIQe, General Electric Healthcare, GE Ultraschall, Deutschland GmbH & Co, Germany) equipped with a linear-array transducer with band frequency 7-12 MHz was used for collecting the images. Gain was set at 48% of the range, Dynamic Range was maintained at 93 dB, and Time Gain Compensation was kept at the same (neutral) position for all imaged depths. The depth setting was adjusted for each muscle in order to visualize their superior and inferior margins. Images were recorded as DICOM files and stored in a personal computer for later processing.

MT and EI were obtained using Image J (National Institutes of Health, Bethesda, MD, USA) by the same examiner. The three images of each muscle for the two sides and

from both data-collecting sessions and for transverse and longitudinal views from the twenty participants were analysed, in a total of 1824 images.

MT was measured as the largest distance between the superficial and deep fasciae, identified by their hyperechoic appearance. Two different ROIs were selected to measure EI: (1) maximum ROI, draw for each scan to include as much of the muscle as possible, avoiding bone and surrounding fasciae (Figure 12); (2) small ROI, a 70 mm^2 ROI positioned over the central region of the muscle image (Figure 12). EI was then defined as the mean level of gray within the ROI in 8-bit resolution images (gray levels from 0 to 255, where black = 0 and white = 255).

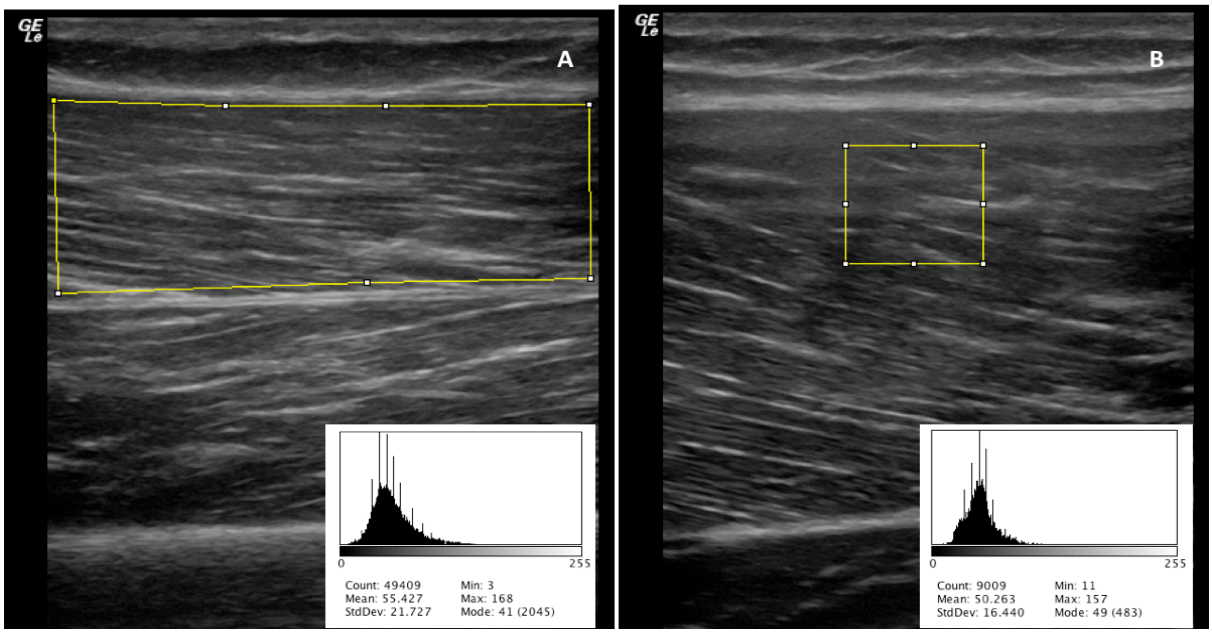


Figure 12: Ultrasound images of two different ROIs used to measure muscle echo-intensity. (A) A maximum ROI was defined for each image to include as much of the muscle as possible, avoiding bone and surrounding fasciae. (B) A smaller rectangular ROI with a set size of 70 mm^2 was positioned approximately at the centre of the muscle image.

Statistical analysis

Intra- and inter-session reliability for MT and EI were assessed using intra-class correlation coefficient (ICC) ($_{3,1}$; method: alpha, two-way mixed, consistency). For inter-session reliability, the average of the three measures obtained in each session was used.

Standard error of measurement (SEM) and smallest detectable change (SDC) were also calculated. SEM indicates the precision of the measurement and was calculated based on the ICC and the SD of the mean of the differences between the two measurements

(i.e., SEM = SD$\sqrt{}$1-ICC). The SDC was based on the SEM, using the formula: SDC = 1.96* $\sqrt{}$2*SEM.

The level of agreement between transverse and longitudinal scans and between the two ROI sizes was evaluated by Bland-Altman analysis and respective 95% limits of agreement (LoA), using the data collected in the first session. In Bland-Altman plots, the difference between the mean of the three images belonging to one measure was plotted against the overall mean [i.e., mean of 3 x 2 images (3 images for each of 2 measurement conditions)]. The t-test was used for pairwise comparisons.

Data are reported as mean ± standard deviation (SD). Threshold for statistical significance was set at $p<0.05$. All analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package.

2.1.2 Results

Data of MT for each of the quadriceps femoris heads are presented in Table 3. VM and VI showed the largest and the smallest MT, respectively, either when measured on transverse or longitudinal scans and for both right and left sides. The right VM, VL, and RF, in transverse scans, and VM and RF, in longitudinal scans, presented larger MT values than those on the left side ($p<0.05$; Table 3). MT was larger on transverse scans compared with longitudinal scans for all muscle heads ($p<0.05$; Table 3) except for right VM, left VM and left VI.

Table 3: Data of muscle thickness for the four heads of the left and right quadriceps femoris.

Muscle	Muscle thickness (cm)			
	First Session		Second Session	
	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan
Right Side				
Vastus Medialis	2,7±0.6[a]	2.5±0.4	2,6±0.4	2.4±0.4
Vastus Lateralis	2.2±0.3[a,b]	2.1±0.3[a]	2.2±0.4	2.1±0.4
Rectus Femoris	2.4±0.4[a,b]	2.3±0.4[a]	2.3±0.4	2.3±0.4
Vastus Intermedius	1.7±0.4[b,c]	1.7±0.4	1.5±0.3	1.5±0.3
Left Side				
Vastus Medialis	2.8±0.4	2.4±0.3	2.8±0.5	2.4±0.4
Vastus Lateralis	2.1±0.2[b]	2.0±0.2	2.1±0.2	2.0±0.2
Rectus Femoris	2.2±0.4[b]	2.1±0.4	2.2±0.4	2.1±0.4
Vastus Intermedius	1.7±0.4[b,c]	1.7±0.4	1.6±0.4	1.6±0.3

Pairwise comparison between right and left side and between transverse and longitudinal scans were performed only with data from the first session. Data presented as mean ± SD.

[a] Significant difference between the two sides (right thigh vs. left thigh; $p<0.05$).

[b] Significant difference between the two scans (transverse vs. longitudinal; $p<0.05$).

[c] Significant difference between two sessions ($p<0.05$).

Table 4 presents the data for EI. The lowest EI values were registered in VI, whereas the VL showed the highest EI values of all muscles. For right VM and VL, and left VM, VL and RF, EI values were significantly different between the two ROIs (p<0.05; Table 4). Also, significant differences in EI were found between transverse and longitudinal scans for the same muscles except the left VM (p<0.05; Table 4). Differences between the right and left sides in muscle EI were only observed in VM (p<0.05; Table 4).

Table 4: Data of muscle echo-intensity for the four heads of the left and right quadriceps femoris using maximum and rectangular ROIs.

Muscle	Muscle echo-intensity (mean pixel value) (a.u.)							
	MAXIMUM ROI				RECTANGULAR ROI			
	First Session		Second Session		First Session		Second Session	
	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan
Right Side								
Vastus Medialis	47.3±5.8[a,b,c]	49.1±5.3	46.9±4.7	49.2±5.2	63.2±9.2	54.0±7.1	59.7±5.4	53.8±6.9
Vastus Lateralis	51.9±5.8[b,c]	53.9±6.2	51.6±6.8	53.2±7.0	59.9±7.7	59.1±6.9	56.8±9.3	56.4±7.7
Rectus Femoris	45.1±4.6	44.7±5.4	45.2±6.1	45.0±7.3	48.5±8.2	49.1±8.2	46.9±7.8	48.2±11.1
Vastus Intermedius	27.5±7.3	29.7±8.7	29.5±6.5	31.0±8.4	31.3±13.1	31.5±11.4	37.3±9.6	35.5±8.5
Left Side								
Vastus Medialis	45.07±5.4[c]	50.2±5.8	49.2±5.8	49.0±6.4	63.9±8.2	57.0±6.4	60.5±7.2	53.3±8.4
Vastus Lateralis	53.0±6.5[b,c]	56.6±7.6	53.0±6.8	53.9±7.8	59.4±7.8	60.8±8.9	57.5±9.4	56.7±9.4
Rectus Femoris	44.2±5.6[b,c]	46.8±8.2	44.4±7.2	46.2±7.6	48.6±8.2	51.0±8.4	45.9±10.3	49.2±9.4
Vastus Intermedius	27.2±7.9	28.5±10.2	29.0±7.1	31.0±10.0	31.3±13.1	29.9±11.8	34.5±10.1	34.0±11.2

Pairwise comparison between right and left side and between transverse and longitudinal scans were performed only with data from the first session.
Data presented as mean ± SD

[a] Significant difference between the two sides (right thigh vs. left thigh; p<0.05).

[b] Significant difference between the two scans (transverse vs. longitudinal; p<0.05).

[c] Significant difference between two ROIs (maximum ROI and rectangular ROI; p<0.05).

Tables 5, 6 and 7 report reliability data for MT and EI measures. High to very high intra-session ICCs were found for MT measures with just three ICC values under 0.80 (Table 5). Inter-session ICCs were also high to very high with only one value below 0.70 (left VL in longitudinal scan; Table 5). SEM values for MT were similar across the four heads of the quadriceps femoris, ranging between 0.07 cm and 0.19 cm. SDC values varied between 0.19 cm (VM, transverse scan; Table 5) and 0.53 cm (VI, longitudinal scan; Table 5). Measures from longitudinal scans tended to show higher SDC values compared to those obtained from transverse muscle views. Intra-session ICC values for EI were also high to very high with maximum ROI (Table 6).

Reliability of EI values was also high when using a smaller rectangular ROI, although in this case a few values were between 0.70 and 0.80 (Table 7). Inter-session ICC values for EI using maximum ROI were generally above 0.70, except for right RF and VI and left RF (Table 6). When using the maximum ROI, SDC values for muscle EI were consistently smaller for VM and larger in VI (Table 6). The number of ICC values below the 0.70

acceptable level increased when the smaller rectangular ROI was used (Table 7). Likewise, larger SDC values for muscle EI were found when using the latter ROI (Table 7).

Table 5: Reliability of muscle thickness measures: ICC, SEM, and SDC values.

Muscle	Muscle thickness									
	First Session		Second Session		Inter-Session					
	ICC Consistency		ICC Consistency		ICC Consistency		SEM Consistency (cm)		SDC (cm)	
	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan
Right Side										
Vastus Medialis	0.98	0.97	0.98	0.97	0.98	0.90	0.09	0.13	0.24	0.37
Vastus Lateralis	0.98	0.98	0.98	0.99	0.81	0.82	0.15	0.15	0.43	0.43
Rectus Femoris	0.99	0.98	0.98	0.91	0.92	0.91	0.12	0.13	0.34	0.37
Vastus Intermedius	0.97	0.98	0.97	0.98	0.89	0.74	0.13	0.19	0.35	0.53
Left Side										
Vastus Medialis	0.86	0.88	0.97	0.95	0.96	0.80	0.07	0.16	0.19	0.43
Vastus Lateralis	0.89	0.94	0.82	0.95	0.81	0.70	0.09	0.14	0.25	0.38
Rectus Femoris	0.99	0.98	0.98	0.99	0.96	0.88	0.09	0.14	0.24	0.39
Vastus Intermedius	0.98	0.99	0.96	0.98	0.86	0.79	0.15	0.17	0.41	0.46

ICC= intra-class correlation coefficient; SEM= standard error of measurement; SDC= smaller detectable change.

Table 6: Reliability of muscle echo-intensity measures for maximum ROI: ICC, SEM, and SDC values.

Muscle	Muscle echo-intensity (mean pixel value)- maximum ROI									
	First Session		Second Session		Inter-Session					
	ICC Consistency		ICC Consistency		ICC Consistency		SEM Consistency (a.u.)		SDC (a.u.)	
	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan
Right Side										
Vastus Medialis	0.87	0.90	0.86	0.89	0.84	0.94	2.15	1.34	5.96	3.72
Vastus Lateralis	0.92	0.94	0.90	0.92	0.83	0.69	2.61	3.70	7.23	10.25
Rectus Femoris	0.90	0.89	0.91	0.95	0.86	0.72	2.06	3.37	5.72	9.35
Vastus Intermedius	0.97	0.93	0.93	0.93	0.62	0.72	4.26	4.51	11.82	12.50
Left Side										
Vastus Medialis	0.86	0.85	0.89	0.95	0.81	0.90	2.42	1.95	6.70	5.41
Vastus Lateralis	0.92	0.94	0.88	0.96	0.91	0.83	2.04	3.21	5.64	8.90
Rectus Femoris	0.88	0.95	0.92	0.96	0.69	0.83	3.61	3.24	10.00	8.98
Vastus Intermedius	0.96	0.96	0.92	0.97	0.84	0.84	3.03	4.00	8.41	11.09

ICC= intra-class correlation coefficient; SEM= standard error of measurement; SDC= smaller detectable change

Table 7: Reliability of muscle echo-intensity measures for rectangular ROI: ICC, SEM, SDC.

Muscle	Muscle echo-intensity (mean pixel value) - rectangular ROI									
	First Session		Second Session		Inter sessions					
	ICC Consistency		ICC Consistency		ICC Consistency		SEM Consistency (a.u.)		SDC (a.u.)	
	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan	Transverse scan	Longitudinal scan
Right Side										
Vastus Medialis	0.82	0.74	0.80	0.76	0.56	0.79	5.44	2.76	15.09	7.66
Vastus Lateralis	0.86	0.83	0.87	0.88	0.79	0.53	5.59	5.03	15.48	13.96
Rectus Femoris	0.83	0.84	0.83	0.94	0.55	0.68	6.13	5.28	16.99	14.64
Vastus Intermedius	0.92	0.95	0.94	0.84	0.60	0.56	5.78	6.70	16.01	18.56
Left Side										
Vastus Medialis	0.88	0.78	0.85	0.87	0.82	0.58	3.33	4.86	9.23	13.48
Vastus Lateralis	0.77	0.87	0.89	0.93	0.80	0.73	3.91	4.86	10.84	13.47
Rectus Femoris	0.82	0.86	0.87	0.77	0.48	0.74	6.71	4.53	18.60	12.54
Vastus Intermedius	0.93	0.87	0.90	0.97	0.71	0.73	6.34	6.04	17.58	16.74

ICC= intra-class correlation coefficient; SEM= standard error of measurement; SDC= smaller detectable change

Bland-Altman plots showed close to zero bias for MT measures both in transverse and longitudinal scans (range: -0.04-0.17 cm; Table 8, Figure 13) with upper 95% LoA in the range 0.13-1.70 cm, and lower 95% LoA in the range -0.09 to -1.22 cm (Table 8, Figure 13).

Table 8: Summary of differences (first session only) between transverse and longitudinal scans (MT and EI) and between maximum ROI and rectangular ROI (EI only).

Muscle	Muscle thickness (cm)				Echo-intensity (a.u.)							
	Difference between transverse and longitudinal scans				Difference between transverse and longitudinal scans				Difference between maximum ROI and rectangular ROI			
	Mean	SD	LoA		Mean	SD	LoA		Mean	SD	LoA	
			Upper	Lower			Upper	Lower			Upper	Lower
Right Side												
Vastus Medialis	0.20	0.75	1.70	-1.22	-1.10	3.87	6.50	-8.70	-15.10	5.142	-4.48	-25.71
Vastus Lateralis	0.22	0.12	0.36	-0.12	-2.66	3.15	3.53	-8.84	-7.60	3.96	0.16	-15.36
Rectus Femoris	0.04	0.07	0.17	-0.09	-1.31	3.13	4.84	-7.45	-5.38	4.602	3.64	-14.40
Vastus Intermedius	-0.02	0.19	0.35	-0.39	-2.09	4.46	6.65	-10.83	-21.60	11.91	1.74	-44.94
Left Side												
Vastus Medialis	0.03	0.70	1.41	-0.67	0.81	4.72	10.05	-8.44	-12.64	6.44	-0.02	-25.26
Vastus Lateralis	0.17	0.17	0.50	-0.17	-4.34	4.41	4.29	-12.97	-7.029	4.67	2.12	-16.18
Rectus Femoris	0.11	0.13	0.36	-0.15	-1.86	5.08	8.11	-11.83	-3.81	4.61	5.23	-12.85
Vastus Intermedius	-0.04	0.09	0.13	-0.21	-1.71	5.12	8.32	-11.74	-9.09	6.43	3.51	-21.69

Differences are calculated as: transverse scan measure minus longitudinal scan measure; maximum ROI measure minus rectangular ROI measure.
LoA= Limits of Agreement (95%)

For EI measures, Bland-Altman plots showed a small bias when measures were collected from transverse vs. longitudinal scans (upper 95% LoA for quadriceps femoris: -3.53-10.05 a.u.; lower 95% LoA for quadriceps femoris: -12.97 to -7.45, Table 8, Figure 14).

Regarding agreement in EI measures taken with each ROI size, Bland-Altman analysis demonstrated a slight bias, with a tendency for larger EI values when using the rectangular ROI comparing with the maximum ROI (upper 95% LoA for quadriceps femoris: -4.48-5.23 a.u.; lower 95% LoA for quadriceps femoris: -44.94 to -12.85 a.u.) (Table 8, Figure 15).

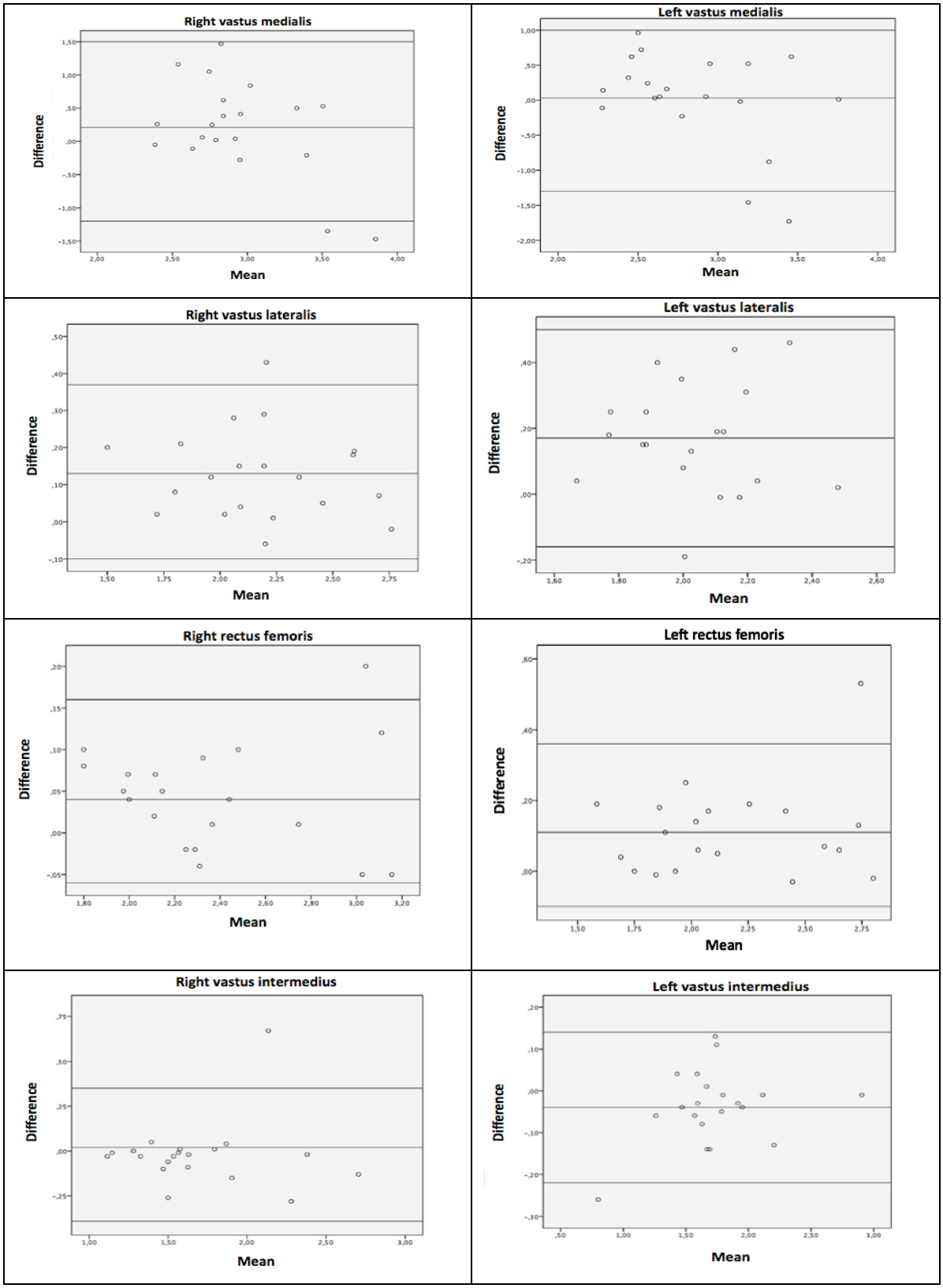


Figure 13: Bland-Altman plots for muscle thickness measures (transverse and longitudinal scans) obtained from two different ultrasound images acquired in the first session and for each side. The horizontal axis represents the mean of the two measurement conditions (transverse and longitudinal scans) and the vertical axis represents the difference from each measurement to the mean. Dotted lines represent the mean difference and the limits of agreement (± 1.96 SD).

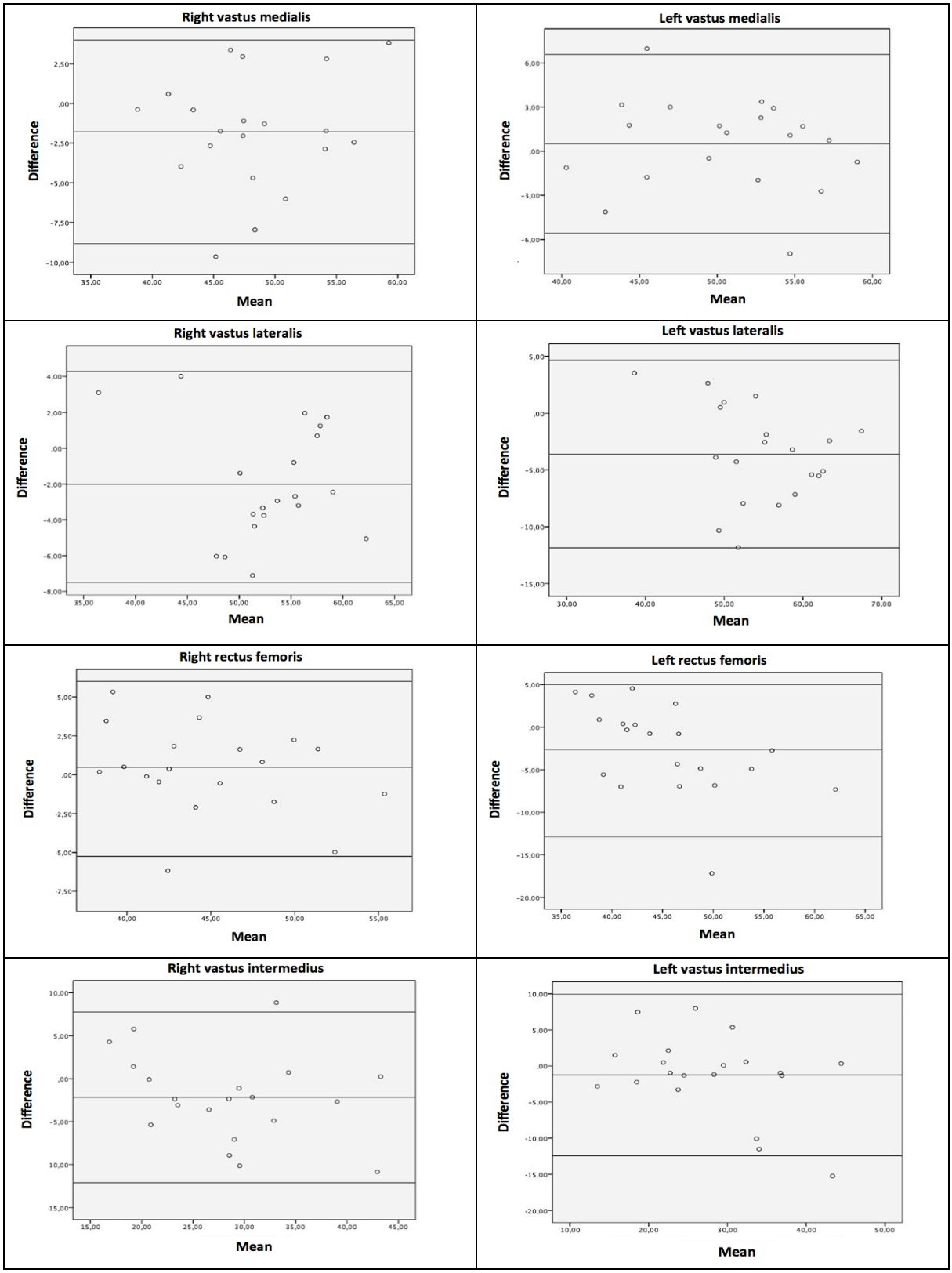


Figure 14: Bland-Altman plots for muscle echo-intensity measured in transverse and longitudinal scans obtained from two different ultrasound images acquired in the first session and for each side. The horizontal axis represents the mean of the two measurement conditions (transverse and longitudinal scans) and the vertical axis represents the difference from each measurement to the mean. Dotted lines represent the mean difference and the limits of agreement (± 1.96 SD).

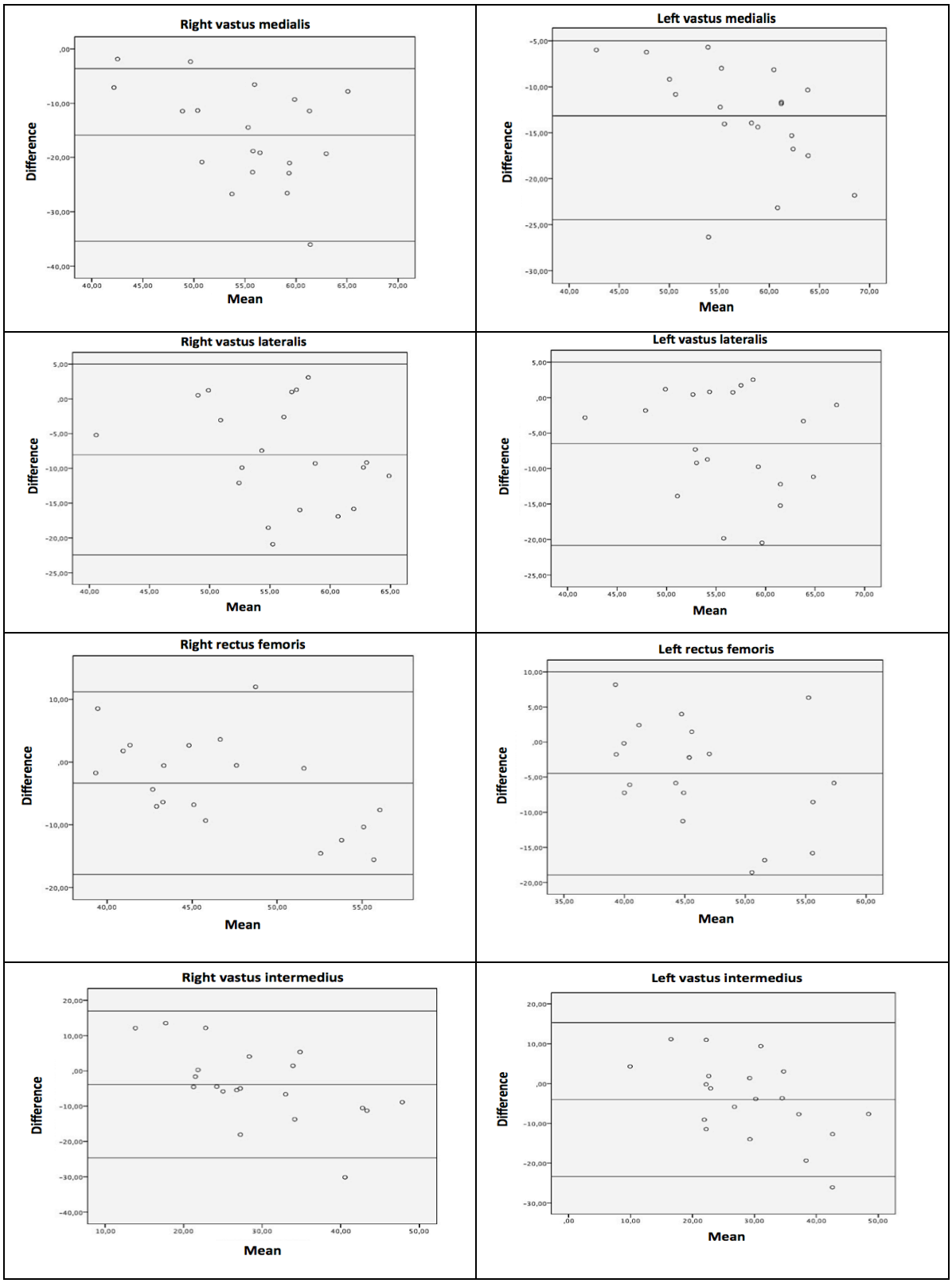


Figure 15: Bland-Altman plots for muscle echo-intensity measured by two different ROIs (maximum ROI and rectangular ROI) in the first session and for each side. The horizontal axis represents the mean of the two measurement conditions (maximum and rectangular ROIs) and the vertical axis represents the difference from each measurement to the mean. Dotted lines represent the mean difference and the limits of agreement (± 1.96 SD).

2.1.3 Discussion

In this study, a high to very high intra- and inter-session reliability for MT and EI measures could be demonstrated. Just in few cases was inter-session ICC for muscle EI below the acceptable 0.70 level.

MT values in the quadriceps femoris varied between 1.5 and 2.8 cm, respectively for VI and VM. This is in line with reported MT values of around 2-2.5 cm for the VL (Caresio et al., 2014; Boer et al., 2008), and of 2.4 cm and 1.8 cm for VM and VI, respectively (Blazevich et al., 2006). Other studies, using the inside edges of the RF and VI muscle borders as a reference, report MT values of around 3.9 cm (Agyapong-Badu et al., 2014) and 3.6 cm (Fukumoto et al., 2012), which are similar to the sum of RF and VI MT in our study.

Muscle EI values might offer important insight into muscle changes caused by disease (Jenkins et al, 2015) in part because it is more objective and possibly more reliable than simple visual assessment of ultrasound images (Pillen, 2010). We could find considerable differences in EI across the quadriceps femoris heads, with VL showing the highest EI (mean gray level range: 51.6-56.6 a.u.) and VI the lowest ones (mean gray level range: 27.2-31.0 a.u). Caresio, et al. (2014) reported EI values for VL (mean gray level 48.9 ± 6.9 a.u.) and RF (48.2 ± 5.5 a.u.) close to those found in the present study (Caresio et al., 2015). Studies conducted in older people, report higher muscle EI values in the quadriceps femoris, in some cases approaching a mean gray level of 100 a.u. (Caresio et al., 2014; Fukumoto et al., 2012; Wilhelm et al., 2014). Such high EI probably reflects the effect of ageing and muscle changes, such as an increase in connective and adipose tissue in muscles and diminished muscle mass (i.e., thickness) (Cadore et al., 2012; Fukumoto et al., 2012; Nishihara et al., 2014).

Our results show high to very high intra- and inter-session ICCs for MT. Compared to transverse scans, MT measured in the longitudinal scans are somewhat less reliable, displaying slightly larger SEM and SDC values (see Table 5). Several other studies report very good reliability of ultrasound MT measures. Studies by Palmer et al. (2015) and Mangine et al. (2014) report inter-session ICCs for RF and VL MT in transverse scans between 0.96 and 0.89 (Mangine, 2014; Palmer, 2015). Also, Raj et al. (2012) reported ICCs of 0.96 to 0.97 for measures of VL thickness in longitudinal scans taken with seven days interval in a group of older adults (Raj et al., 2012). Lastly, Strasser et al. (2013) demonstrated a high reliability of ultrasound MT measurements for the several heads of the quadriceps femoris in young and old participants, with ICCs between 0.85 and 0.97 (Strasser et al., 2013). In our study, we also derived SEM and SDC values in order to

assess reliability. Our SEM values for MT measurements were similar across the whole quadriceps femoris, ranging from 0.07 cm to 0.19 cm. However, the SDC values for MT measures showed considerable variation across this muscle. In this case, the smallest SDC value was 0.19 cm, for left VM thickness obtained from transverse scans. The largest SDC reached 0.53 cm and was found for MT measures belonging to the right VI scanned longitudinally. Such SDC values correspond to around 7 and 30% of the respective mean MT value. Reported SEM values for MT of trunk muscles are comparable to those reported in the present study, although slightly lower (0.02-0.05 cm, transversus abdominis and lumbar multifidus) (Jhu et al., 2010; Koppenhaver et al., 2009). As for SDC of MT measured from longitudinal and transverse ultrasound scans, the reported values vary between 0.07 cm and 0.08 cm (Koppenhaver et al., 2009), and between 0.06 cm and 0.13 cm (Jhu et al., 2010; Koppenhaver et al., 2009). The reliability of our ultrasound MT measures appeared to be slightly better in transverse scans than in longitudinal ones. Transverse scans offer a better visualization of the anatomical details of the several quadriceps femoris heads and based on our data should be used to measure MT. While longitudinal ultrasound scans are particularly useful when studying muscle architecture of limb muscles since the orientation of muscle fascicles is easily viewed in these images, they seem to be less reliable than transverse scans when the goal is to measure MT. Although some authors argue that longitudinal scans should be used for skeletal muscle analysis (Blazevich et al., 2006; Konig et al., 2014; Wilhelm et al., 2014), others advocate the use of transverse views (Agyapong-Badu et al., 2014; Boer et al., 2008; Fukumoto et al., 2012). Although both transverse and longitudinal views seem to be suitable for evaluating MT and muscle EI, differences in precision between the two planes exist and should be taken into account when choosing between the two views.

One of the aims of this study was to determine the effect of ROI size on the reliability of ultrasound muscle EI measures. Our EI measures were highly reliable, both when collected in the same session or in sessions one-week apart. However, and regarding inter-session reliability, ICCs were higher when the whole muscle image was used to obtain muscle EI compared to when a smaller, constant-size rectangular ROI was employed. In the latter case, a number of ICCs did not reach 0.70, which is commonly considered a minimum level for acceptable reliability (Kottner et al., 2011). However, scanning orientation (i.e., transverse or longitudinal) did not affect the reliability of muscle EI measurements in our study. Similar findings were obtained by Caresio et al. (2014) for several upper and lower limb muscles in young adults (Caresio et al., 2015). They report intra-session muscle EI ICCs of 0.54 to 0.86 depending on ROI size, with

larger ROIs being associated with higher reliability (Caresio et al., 2015). Radaelli et al. (2012) also showed less reliability when small ROIs are adopted to determine muscle EI (Radaelli et al., 2012). Fukumoto et al. (2012) also found very high reliability for muscle EI measures for the RF (ICC: 0.91), as well as for other hip and abdominal muscles (ICCs: 0.87–0.99) (Fukumoto et al, 2012). For biceps brachii, ICC values >0.90 were again reported for muscle EI (Chen et al., 2012; Radaelli et al., 2012). Our SDC values for EI ranged from 3.72 to 12.50 a.u. for maximum ROI and from 7.66 to 18.56 a.u. for rectangular ROI. SEM values for two different ROIs for EI were similar (1.34-6.34 a.u.) but rectangular ROI demonstrated larger SEM values. These findings are in line with Caresio et al. (2014) study that shows that a minimum ROI size is required for a reliable analysis of the muscle EI (Caresio et al., 2014). Also, Matta, et al. (2013) found that a small ROI (1 cm^2) is associated with a larger variance of muscle EI data (Matta, 2013).

Bland-Altman analysis was performed to evaluate the agreement between measures taken from transverse and longitudinal scans and between the two ROIs. The results of this study suggest that MT is underestimated by 0.2 cm when measured from longitudinal scans. Despite such bias, the LoA (-0.21 cm and 0.5 cm for the four heads of quadriceps femoris) are small enough to allow MT measurements to be performed in transverse or longitudinal muscle scans, except for VM muscle that showed larger 95% confidence limits of -1.22 cm and 1.70 cm. Only a few studies conducted Bland–Altman analysis (including mean differences) for ultrasound MT measurements (English et al., 2012). However, Raj et al. (2012) referred similar values for VL muscle for two different tests (Raj et al., 2012).

Bland-Altman plot for muscle EI measured in transverse and longitudinal scans and measured with the two ROI sizes demonstrated higher mean differences and higher LoA, when compared with MT. Pooling the data form the four quadriceps femoris muscle heads and from both sides, the Bland-Altman plot of muscle EI measured in transverse and longitudinal scans showed 95% LoA ranging between -12.97 (lowest lower limit: VL) to 10.05 (highest upper limit: VM) and a bias between -4.34 and 0.81 and towards higher EI values in transverse scans than in longitudinal ones, except for left VM muscle. Considering the two ROIs (maximum ROI and rectangular ROI), the mean difference varies between -21.60 to -3.81 a.u. These results suggest that EI is underestimated when measured with a smaller ROI.

2.1.4 Limitations

The major limitation of this study resides on the young age of the participants, which limits the generalizability to other age groups. Few sources of variability, such as the

hydration levels of the participants (Calhoun, 2015) or subtle changes in posture and muscle relaxation between the sessions, might have contributed to the differences in measurements between sessions (Lima et al., 2012). Other factors that are known to negatively affect reliability of ultrasound measurements include inter-session changes in probe location and orientation and in the degree of compression applied with the probe onto the skin (Jhu et al., 2010; Koppenhaver et al., 2009; Lima et al., 2012). Also, EI measures are sensitive to ultrasound settings and these may differ between different ultrasound devices, thus limiting the generalizability of our results to other equipment. Future studies are important to standardize ultrasound settings for different equipment, as well as inter-operator reliability.

2.1.5 Summary

In conclusion, this study showed that MT and EI measurements from the four heads of the quadriceps femoris are highly reproducible in healthy subjects and therefore could be used in the study of quadriceps femoris muscle changes caused by disease or training. The reliability of EI measurements is sensitive to ROI size. In addition, subtle differences in EI and MT could be found between different quadriceps muscle heads (EI of VL and thickness of VM were highest) as well as between the right and left sides. Bland-Altman plots demonstrated near zero values of bias and low 95% LoA for MT but poorer agreement values for EI. SEM and SDC values for quadriceps femoris MT and EI are low and similar to those found in the literature. The high reproducibility of ultrasound MT measurements makes this technique useful for monitoring short-term changes in muscle mass. The somewhat lower reliability of EI measures must be taken into account when planning for future studies.

These findings can contribute for improving the use of ultrasound to study muscle morphology and particularly to assess small changes occurring due to disease, immobility or muscle overuse.

2.1.6 References

Agyapong-Badu, S., Warner, M., Samuel, D., Narici, M., Cooper, C., & Stokes, M. (2014). Anterior thigh composition measured using ultrasound imaging to quantify relative thickness of muscle and non-contractile tissue: a potential biomarker for musculoskeletal health. *Physiological Measurement*, *35*(10), 2165–76.

Ahtiainen, J., Pakarinen, A., Alen, M., Kraemer, W., & Hakkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *Eur J Appl Physiol*, *89*(6), 555–563.

Alqahtani, M. (2010). *Quantitative Ultrasound Image Analysis of the Gastrocnemius Muscle for Injury Evaluation*. Cardiff University.

Anderson, T., & McDicken, W. (2002). The difference between Colour Doppler Velocity Imaging and Power Doppler Imaging. *Eur J Echocardiogr*, *3*(3), 240–244.

Andreassen, C., Jakobsen, J., & Andersen, H. (2006). Muscle weakness: a progressive late complication in diabetic distal symmetric polyneuropathy. *Diabetes*, *55*(3), 806–812.

Baldwin, C., Paratz, J., & Bersten, A. (2011). Diaphragm and peripheral muscle thickness on ultrasound: Intra-rater reliability and variability of a methodology using non-standard recumbent positions. *Respirology*, *16*(7), 1136–1143.

Bernard, S., LeBlanc, P., Whittom, F., Carrier, G., Jobin, J., Belleau, R., & Maltais, F. (1998). Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*, *158*(2), 629–634.

Blazevich, A., Gill, N., & Zhou, S. (2006). Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *Journal of Anatomy*, *209*(3), 289–310.

Bunnell, A., Ney, J., Gellhorn, A., & Hough, C. (2015). Quantitative neuromuscular ultrasound in intensive care unit-acquired weakness: A systematic review. *Muscle & Nerve*, *52*(5), 701–708.

Cadore, E., Izquierdo, M., Conceição, M., Radaelli, R., Pinto, R., Baroni, B., … Kruel, L. (2012). Echo intensity is associated with skeletal muscle power and cardiovascular performance in elderly men. *Experimental Gerontology*, *47*(6), 473–8.

Calhoun, M. (2015). *Validity of ultrasound velocity to detect changes in the hydration status of male and female athletes during acute dehydration and rehydration. Department of Exercise Science*. Graduated School at Appalachian State University.

Caresio, C., Molinari, F., Emanuel, G., & Minetto, M. (2015). Muscle echo intensity: Reliability and conditioning factors. *Clinical Physiology and Functional Imaging*, *September*(5), 1–11.

Chen, X., Zheng, Y., Guo, J., Zhu, Z., Chan, S., & Zhang, Z. (2012). Sonomyographic responses during voluntary isometric ramp contraction of the human rectus femoris muscle. *European Journal of Applied Physiology*, *112*(7), 2603–14.

Cohen, E., Mier, A., Heywood, P., Murphy, K., Boultbee, J., & Guz, A. (1994). Excursion-volume relation of the right hemidiaphragm measured by ultrasonography and respiratory airflow measurements. *Thorax*, *49*(9), 885–889.

Boer, M., Seynnes, O., Prampero, P., Pisot, R., Mekjavić, I., Biolo, G., & Narici, M. (2008). Effect of 5 weeks horizontal bed rest on human muscle thickness and architecture of weight bearing and non-weight bearing muscles. *European Journal of Applied Physiology*, *104*(2), 401–7.

English, C., Fisher, L., & Thoirs, K. (2012). Reliability of real-time ultrasound for measuring skeletal muscle size in human limbs in vivo: a systematic review. *Clinical Rehabilitation*, *26*(10), 934–944.

Frontera, W., Hughes, V., Fielding, R., Fiatarone, M., Evans, W., & Roubenoff, R. (2000). Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol*, *88*(4), 1321–1326.

Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., … Ichihashi, N. (2012). Skeletal muscle quality assessed from echo intensity is associated with muscle

strength of middle-aged and elderly persons. *European Journal of Applied Physiology*, *112*(4), 1519–25.

Häkkinen, K., Pakarinen, Alen, M., Kauhanen, H., & Komi, P. (1988). Neuromuscular and hormonal adaptations in athletes to strength training in two years. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *65*(6), 2406–2412.

Hammond, K., Mampilly, J., Laghi, F., Goyal, A., Collins, E., McBurney, C., … Tobin, M. (2014). Validity and reliability of rectus femoris ultrasound measurements: Comparison of curved-array and linear-array transducers. *Journal of Rehabilitation Research and Development*, *51*(7), 1155–1164.

Jenkins, N.; Miller, J.; Buckner, S.; Cochrante, K.; Bergstrom, H.; Hill, E.; Smith, C.; Housh, T.; Cramer, J. (2015). Test–retest reliability of single transverse versus panoramic ultrasound imaging for muscle size and echo intensity of the biceps brachii. *Ultrasound in Medicine and Biology*, *41*(6), 1584–1591.

Jhu, J., Chai, H., Jan, M., Wang, C., Shau, Y., & Wang, S. (2010). Reliability and Relationship Between 2 Measurements of Transversus Abdominis Dimension Taken During an Abdominal Drawing-in Maneuver Using a Novel Approach of Ultrasound Imaging. *Journal of Orthopaedic & Sports Physical Therapy*, *40*(12), 826–832.

Konig, N., Cassel, M., Intziegianni, K., & Mayer, F. (2014). Inter-Rater Reliability and Measurement Error of Sonographic Muscle Architecture Assessments. *Journal of Ultrasound in Medicine*, *33*(5), 769–777.

Koppenhaver, S., Hebert, J., Fritz, J., Parent, E., Teyhen, D., & Magel, J. (2009). Reliability of rehabilitative ultrasound imaging of the transversus abdominis and lumbar multifidus muscles. *Archives of Physical Medicine and Rehabilitation*, *90*(1), 87–94.

Kottner, J., Audig, L., Brorson, S., Donner, A., & Gajewski, B. (2011). Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. *Journal of Clinical Epidemiology*, *64*, 96–106.

Lafortuna, C., Maffiuletti, N., Agosti, F., & Sartorio, A. (2005). Gender variations of body composition, muscle strength and power output in morbid obesity. *International Journal of Obesity*, *29*(7), 833–841.

Lima, K., Matta, T., & de Oliveira, L. (2012). Reliability of the rectus femoris muscle cross-sectional area measurements by ultrasonography. *Clinical Physiology and Functional Imaging*, *32*(3), 221–226.

Mangine, G., Hoffman, J., Gonzalez, A., Jajtner, A., Scanlon, T., Rogowski, J., … Jeffrey R. (2014). Bilateral Differences in Muscle Architecture and Increased Rate of Injury in National Basketball Association Players. *Journal of Athletic Training*, *49*(6), 794–799.

Marimuthu, K., Murton, A., & Greenhaff, P. (2011). Mechanisms regulating muscle mass during disuse atrophy and rehabilitation in humans. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *110*(2), 555–560.

Matta, T., Oliveira, V., Pereira, W., Oliveira, L. (2013). Variability and Reliability of Elbow Flexors and Rectus Femoris Echo Intensity in healthy adults. *XXIV Congress of the International Society of Biomechanics*, 3–4.

McMeeken, J., Beith, I., Newham, D., Milligan, P., & Critchley, D. (2004). The relationship between EMG and change in thickness of transversus abdominis. *Clinical Biomechanics*, *19*(4), 337–342.

Nishihara, K., Kawai, H., Hayashi, H., Naruse, H., Kimura, A., Gomi, T., & Hoshi, F. (2014). Frequency analysis of ultrasonic echo intensities of the skeletal muscle in elderly and young individuals. *Clinical Interventions in Aging*, *9*, 1471–1478.

Norman, K., Schütz, T., Kemps, M., Josef Lübke, H., Lochs, H., & Pirlich, M. (2005). The Subjective Global Assessment reliably identifies malnutrition-related muscle dysfunction. *Clinical Nutrition (Edinburgh, Scotland)*, *24*(1), 143–50.

O'Sullivan, C., McCarthy Persson, U., Blake, C., & Stokes, M. (2012). Rehabilitative ultrasound

measurement of trapezius muscle contractile states in people with mild shoulder pain. *Manual Therapy*, *17*(2), 139–144.

Palmer, T., Akehi, K., Thiele, R., Smith, D., Thompson, B. (2015). Reliability of Panoramic Ultrasound Imaging in Simultaneously Examining Muscle Size and Quality of the Hamstring Muscles in Young, Healthy Males and Females. *Ultrasound in Medicine & Biology*, *41*(3), 675–684.

Pasta, G., Nanni, G., Molini, L., & Bianchi, S. (2010). Sonography of the quadriceps muscle: Examination technique, normal anatomy, and traumatic lesions. *Journal of Ultrasound*, *13*(2), 76–84.

Pillen, S. (2010). Skeletal muscle ultrasound. *European Journal Translational Myology*, *1*(4), 145–155.

Pouch, A., Cary, T., Schultz, S., & Sehgal, C. (2010). In vivo noninvasive temperature measurement by B-mode ultrasound imaging. *Journal of Ultrasound in Medicine : Official Journal of the American Institute of Ultrasound in Medicine*, *29*(11), 1595–1606.

Radaelli, R., Bottaro, M., Wilhelm, E., Wagner, D., & Pinto, R. (2012). Time Course of Strength and Echo Intensity Recovery After Resistance Exercise in Women. *Journal of Strength and Conditioning Research*, *26*(9), 2577–2584.

Raj, I., Bird, S., & Shield, A. (2012). Reliability of ultrasonographic measurement of the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults. *Clinical Physiology and Functional Imaging*, *32*(1), 65–70.

Roubenoff, R., & Hughes, V. (2000). Sarcopenia: Current Concepts. *J Gerontol A Biol Sci Med Sci*, *55*(12), M716-724.

Strasser, E., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–2388.

Swallow, E., Reyes, D., Hopkinson, N., Man, W., Porcher, R., Cetti, E. J., … Polkey, M. (2007). Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. *Thorax*, *62*(2), 115–120.

Vlychou, M., & Teh, J. (2008). Ultrasound of muscle. *Current Problems in Diagnostic Radiology*, *37*(5), 219–230.

Wallwork, T., Hides, J., & Stanton, W. (2007). Intrarater and interrater reliability of assessment of lumbar multifidus muscle thickness using rehabilitative ultrasound imaging. *The Journal of Orthopaedic and Sports Physical Therapy*, *37*(10), 608–612.

Wilhelm, E., Rech, A., Minozzo, F., Botton, C., Radaelli, R., Teixeira, B., … Pinto, R. (2014). Concurrent strength and endurance training exercise sequence does not affect neuromuscular adaptations in older men. *Experimental Gerontology*, *60*, 207–214.

Wilhelm, E., Rech, A., Minozzo, F., Radaelli, R., Botton, C., & Pinto, R. (2014). Relationship between quadriceps femoris echo intensity, muscle power, and functional capacity of older men. *Age*, *4*(Fev 11), 1–6.

Wong, A., Parent, E., & Kawchuk, G. (2013). Reliability of 2 ultrasonic imaging analysis methods in quantifying lumbar multifidus thickness. *The Journal of Orthopaedic and Sports Physical Therapy*, *43*(4), 251–62.

Zbojniewicz, A. (2014). US for Diagnosis of Musculoskeletal Conditions in the Young Athlete: Emphasis on Dynamic Assessment. *RadioGraphics*, *34*(5), 1145–1162.

Chapter 3: Evaluation of muscle adaptations to strength training by ultrasound imaging

From a morphological point of view, there is still no total agreement about the muscular changes that a particular training program may bring (Franchi, 2014; Blazevich, 2007). Also, the full potential of imaging to investigate the muscle adaptations to different types of stimuli related to different kinds of strength training is still unknown. So, the aim of this Chapter is to investigate the changes in muscle size (i.e., MT) and muscle quality (muscle stiffness/hardness and EI) in response to a strength training program.

This Chapter is divided into two studies. The first one addresses the morphological changes affecting the four heads of the quadriceps femoris in young adults when submitted to 15 weeks of strength training. For this purpose, EI and MT were chosen to characterize the quadriceps femoris adaptations to the strength training program.

The second study reports data from QSE (the first generation of elastography) as a means to evaluate changes in VL's muscle stiffness during the same training program.

3.1 Muscle thickness and echo-intensity changes of the quadriceps femoris muscle during a strength training program

MSK ultrasound is increasingly important both for diagnosis and research (O'Neil, 2008). Ultrasound imaging is a modality with numerous advantages: it is non-invasive, low cost, easy accessible, and does not use ionizing radiation (Pillen, 2010; Vlychou & Teh, 2008). Although the dependency on the operator is a disadvantage of US imaging, this is surpassed by the advantages of this technique so that is regarded as an alternative or a complement to magnetic resonance imaging (Gabrielle et al., 2013). Ultrasound imaging is used for MSK examination in order to assess morphological changes, mainly changes in thickness and EI of skeletal muscles (Nielsen et al., 2006). Measurements of these parameters can then be analysed and related to muscle function (Ruas et al., 2017).

The effect of strength training and/or regular exercise on the trained muscles can lead to changes in MT and muscle EI. Echo-intensity is defined as the ability of a tissue to reflect the US waves and produce echo, and may contain information of muscle tissue composition (Caresio et al., 2014; Nielsen et al., 2006). When the US beam traverses more easily through the structure, fewer echoes are reflected and the tissue is shown as a hypoechoic structure (e.g., body fluids and low density tissues) (Asakawa et al., 2000). When the opposite occurs, i.e., the US cannot penetrate the structure, more US echoes are reflected, showing a hyperechoic structure (e.g., fat tissue) (Wilhelm et al., 2014). A healthy muscle is displayed in an US image as an hypoechoic structure with its fascicles bounded by the perimysium (hyperechoic tissue), while an altered muscle tissue might show itself as a more hyperechoic and diffuse structure (Maeda et al., 1998).

Skeletal muscles of more active subjects show, in general, lower EI due to an increase in myocyte size relative to the volume of perimysium tissue, and to higher vascularization (Alqahtani, 2010; Trip et al., 2009; Vlychou & Teh, 2008; Walker et al., 2004). Although neural adaptations prevail during the first weeks of strength training, muscle hypertrophy starts to develop after a few weeks of training and becomes a major reason for improvements in muscle strength and performance in the long term (Norrbrand, 2010). Also, few studies demonstrate, in an elderly population, that a relationship exists between EI values and isometric and isokinetic muscle strength (Cadore, 2012; Fukumoto et al., 2012). Furthermore, in middle-aged and elderly women, muscle EI has been positively associated with muscle strength, independently of age and muscle size (Cadore et al., 2012; Pillen et al., 2009).

Therefore, the aim of this study was to investigate the changes in MT and EI in the four heads of the quadriceps femoris in a group of young participants after 15 weeks of strength training.

3.1.1 Materials and Methods

Participants

Twenty-eight healthy males (mean ± SD; age: 20 ± 3.3 years, height: 1.75 ± 0.05 m, weight: 69.76 ± 6.7 kg) volunteered to participate in this study. Participants were fully informed of the purpose and procedures of the study and signed a written informed consent. The study conformed to the guidelines of the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Human Kinetics.

Participants were separated into a control group (n = 8) and a strength training group (n = 20). Participants in the strength training group were further separated in two groups, one performing concentric training (GCon group; n = 11) and the other eccentric training (GEcc group; n = 9).

Participants were excluded from the study if they sustained any injury of the lower limbs in the past six months, had an orthopaedic condition or had surgery involving the lower limbs.

Strength training program

Participants in the training groups undertook 15 weeks of strength training (3 weekly sessions), with a total of 45 training sessions. All participants attended at least 42 out of the 45 planned training sessions (compliance rate = 93.3%). The strength training was conducted on an isokinetic dynamometer (Biodex Medical Systems) and targeted both knee extensors and flexors.

The training protocol consisted on performing maximal concentric (group GCon) or eccentric (group GEcc) contractions during knee flexion and extension. One limb was randomly selected for exercising along the entire knee range of motion (ROM) allowed by the dynamometer (from 100 to 0 degrees of knee flexion), whilst the contralateral limb exercised over only a restricted ROM (from 60º to 0º). Each training session began with a warm-up period of approximately 10 minutes, consisting of cycling on a cycle ergometer (Monark Ergomedic 894E), with a workload of 75-80 W, or running on a treadmill (h / w / Cosmos - Pulsar 3p 4.0) with self-selected velocity (8 to 12 $km.h^{-1}$). The warm up finished with 3-5 knee extension contractions performed on the isokinetic dynamometer at a submaximal intensity.

The concentric training was performed in isokinetic (Biodex System 3 or Biodex System 2) (Figure 16) mode by selecting a pre programmed isokinetic knee extension/flexion protocol. The protocol established the number of contraction sets, the angular velocities, the number of repetitions, and the resting duration between sets. Participants were asked to perform the contractions as strong and as fast as possible throughout the set ROM.

The eccentric training had the same number of contraction sets, contraction repetitions, resting duration, and angular velocities used in the Conc training. During Ecc training, participants were also asked to perform Ecc contractions with their knee flexors, thus avoiding any Conc contractions with the knee extensor muscles, ensuring a purely Ecc training.

The training program included five progression phases. During the first 3 weeks, 5 x 6 repetitions of isokinetic knee extensions were completed at an angular velocity of 60º.s^{-1}. With the limb exercising with shorter ROM, the number of repetitions was increased to 10. During the following 12 weeks, the number of contraction sets performed at 60º.s^{-1} decreased to only 2 but additional sets were performed at 90º.s^{-1} (weeks 4-6), 120º.s^{-1} (weeks 7-9), 150º.s^{-1} (weeks 10-12), and 180º.s^{-1} (weeks 13-15). The number of sets performed at the higher angular velocities increased along the training weeks from 5 up to 7. For the limb training with restricted ROM, the number of contraction sets and repetitions were increased in order to match the total time between the two legs spent contracting.

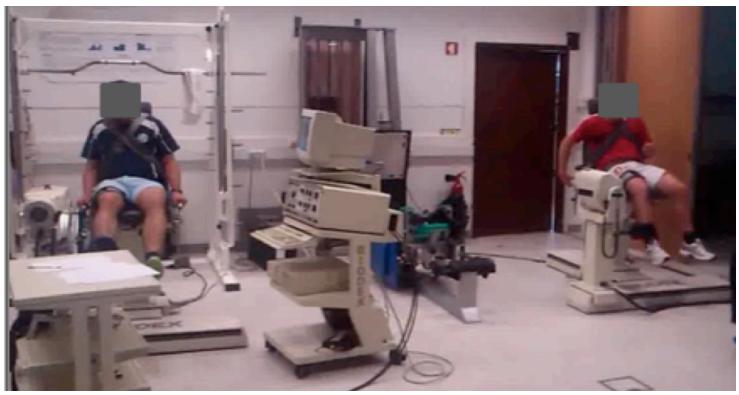


Figure 16: Training unit, with two isokinetic dynamometers – Biodex system. (own source)

Testing of knee extension strength

The strength of the knee extensors was tested on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY, USA) before and after the strength training period. The participants sat on the dynamometer chair with fasten seat belts

crossed in front of the chest and across the pelvis. The tested leg was secured to the arm of the dynamometer by cushioned pads placed immediately above the lateral malleolus and hold in place with Velcro straps. The knee joint was carefully aligned with the rotating axis of the dynamometer arm. Maximal voluntary Isometric contractions (MVC) with the knee extensors were repeated in 5º steps between 100º and 60º of knee flexion (complete knee extension: 0º) and in 10º steps between 60º and 30º knee flexion. Maximal isometric contraction had minimal duration of 2 seconds. All contractions were performed randomly and separated by 2 minutes of rest. The relatively short duration of the MVCs and the fact that only one repetition was performed for each knee angle was justified by the need to prevent muscle fatigue. However, a second MVC was done if the investigator or the participant himself considered the effort to be unsatisfactory or its duration was too short. The torque generated during the isometric MVCs was analog-to-digital converted at 16-bits resolution and at a 1 kHz sampling rate (Biopac MP100, Santa Barbara, USA). The maximum knee extension torque produced before and after the strength training period were then recorded and used for analysis.

Ultrasound Evaluation

All participants were submitted to an US evaluation (Figure 17) of the four heads of the quadriceps femoris before, after 6 weeks and at the end of the 15-weeks strength training period. To avoid possible effects related to the daily routine, participants were evaluated at the same time of day in the three sessions.

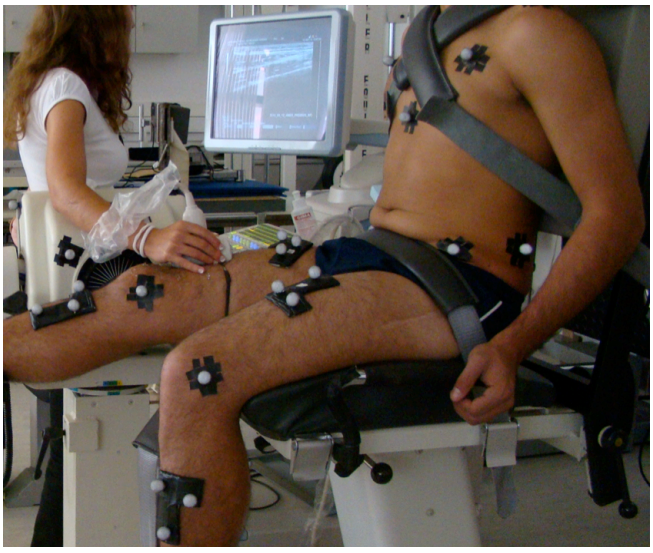


Figure 17: Example of ultrasound images acquisition. (own source)

Scanned muscle regions

Ultrasound images were collected from three different muscle belly sites of the VL, VM, VI, and RF muscles. The places for US scanning were selected according to the scanning map used by Blazevich et al. (2006). For the VM, this corresponded to 5%, 22% and 39% of the distance between the upper edge of the patella and the anterior superior iliac spine, having the upper edge of the patella as the starting point; for the VL the percentages of that distance were 22%, 39% and 56%, and for the VI and RF these percentages were 39%, 56%, 73% (Figure 18).

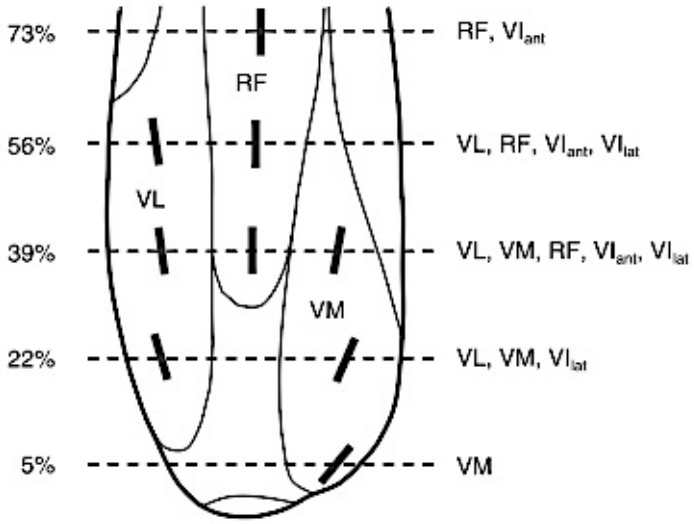


Figure 18: Ultrasound scanning regions for the four heads of the quadriceps femoris according to the Blazevich et al. (2006) protocol. (Blazevich et al. 2006)

Ultrasound protocol

Ultrasound B-mode images of each of the four heads of the quadriceps femoris were acquired in the longitudinal plane with participants seated on the dynamometer chair and with their knees held with 10º flexion. A suitable amount of US coupling gel was used to ensure optimal image quality and to minimize the transducer pressure on the skin. The same examiner, a certified MSK US sonographer, performed the US data collection and analysis.

An US machine (HITACHI EUB 7500, HITACHI Medical Corporation, Tokyo, Japan) with a linear-array transducer with variable frequency band (7-12 MHz) was used to collect the images. All system-setting parameters were optimized individually and for each scanned muscle and then recorded and kept constant during the session. Gain was set at 25% of

the range, dynamic range was maintained at 70 dB, time gain compensation was kept at the same (neutral) position and depth was 65 mm for all images. Compression was minimal and applied in the vertical direction.

Values for EI were obtained using Image J (National Institutes of Health, Bethesda, MD, USA) by the same examiner. A single image for each muscle, each leg, and from each of the three data-collecting sessions was analysed, in a total of 672 images.

For each US scan, a maximum rectangular ROI (Figure 19) was defined manually and always by the same examiner that included as much of the muscle as possible, avoiding bone and surrounding fasciae. The ROI histogram was obtained and the mean pixel value used as a measure of EI.

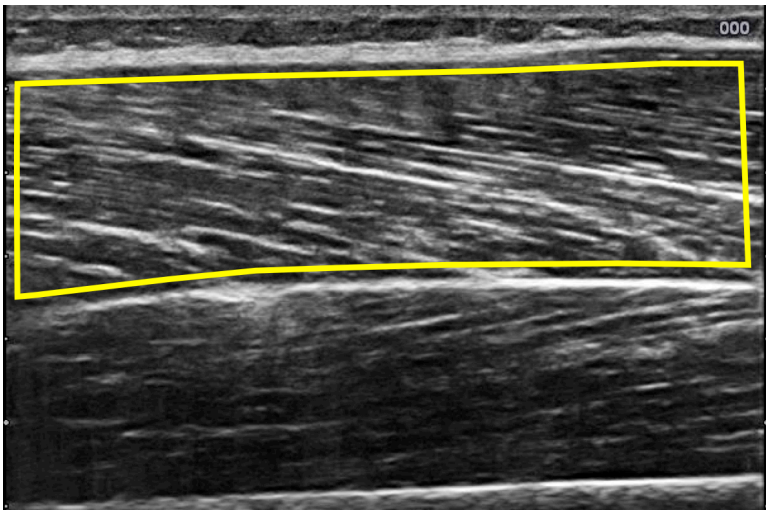


Figure 19: Example of a ROI for determination of EI in a longitudinal scan of the VL.

Statistical analysis

In the Control group, knee muscle strength and US measures were collected only in the beginning and end of the study and, therefore, statistical analyses were conducted separately for this group and the training groups. Mixed two-way analysis of variance (ANOVA) was employed to study the effect of time (3 time points) and of intervention (Ecc vs. Conc and large ROM vs. restricted ROM). The sphericity assumption was assessed with Mauchly's test and the Greenhouse-Geiser correction for the significance level was considered if necessary. Linear regression was used to assess the relationship between MT and EI. Correlations were calculated separately using the data for the mid-portion of the belly of the individual muscles collected before and after training.

Data is reported as mean ± SD. Threshold for statistical significance was set at $p<0.05$. All analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package.

3.1.2 Results

Table 9 shows the data for maximal knee extension torque before and after the 15-weeks strength training program. After strength training, isometric MVC increased significantly in both training groups [$F_{(1,18)}=58.583$; $p<0.001$], but was unchanged in the Con group ($p=0.47$). The increase in maximal knee extension torque was similar in the GCon and GEcc groups as well as in the two limbs (pre-post strength training vs. group interaction: $F_{(1,18)}=0.006$; $p=0.938$; pre-post strength training vs. left limb-right limb interaction: $F_{(1,18)}=0.149$; $p=0.704$).

Table 9: Data for maximal isometric torque produced by the knee extensors before and after strength training for right and left limbs.

	Maximal voluntary isometric torque (Nm)			
	Pre-training		Post-training	
Group	Right limb	Left limb	Right limb	Left limb
Control	303.5±30.8		300.0±35.7	
GCon	300.3±55.2	284.1±46.7	377.3±54.6*	374.3±54.6*
GEcc	241.9±35.0	229.2±12.5	328.9±42.8*	328.9±42.8*

Data presented as mean ± SD.
*Significantly different from pre-training ($p<0.001$)

Muscle thickness

Muscle thickness data for each head of the quadriceps femoris and corresponding to the mid-region of the individual muscles are presented in Table 10. Four participants in the Control group missed the second testing session conducted at the end of 15 weeks. For this reason, data from the Control group at the end of the study are from 4 participants only. No statistical analysis between control and training groups were performed for MT or EI measurements.

Table 10: Data of muscle thickness for each of the four heads of the quadriceps femoris.

Muscle thickness (cm)							
		Baseline		6 weeks		15 weeks	
Group	Muscle	Right side	Left side	Right side	Left side	Right side	Left side
Control	Vastus medialis	2.73±0.10	2.85±0.11	-	-	3.03±0.08	2.75±0.18
	Vastus lateralis	2.40±0.18	2.34±0.07	-	-	2.58±0.01	2.10±0.42
	Rectus femoris	2.29±0.15	2.19±0.03	-	-	2.35±0.10	2.09±0.18
	Vastus intermedius	2.05±0.28	2.3±0.19	-	-	2.15±0.13	1.67±0.19
Concentric training	Vastus medialis	2.70±0.36	2.86±0.43	2.82±0.51	2.71±0.41	2.77±0.40	2.84±0.44
	Vastus lateralis	2.22±0.33	2.23±0.25	2.46±0.30	2.38±0.27	2.48±0.30	2.47±0.27
	Rectus femoris	2.31±0.42	2.29±0.37	2.47±0.41	2.33±0.35	2.50±0.38	2.49±0.34
	Vastus intermedius	1.72±0.41	1.84±0.35	2.28±0.96	2.09±0.37	2.00±0.45	2.13±0.46
Eccentric training	Vastus medialis	2.63±0.32	2.70±0.27	2.85±0.38	2.76±0.37	2.71±0.32	2.87±0.34
	Vastus lateralis	2.05±0.29	1.99±0.30	2.23±0.30	2.24±0.34	2.15±0.29	2.3±0.47
	Rectus femoris	2.17±0.30	2.07±0.28	2.49±0.49	2.43±0.39	2.3±0.41	2.34±0.22
	Vastus intermedius	1.63±0.35	1.74±0.30	2.16±0.41	2.18±0.40	1.94±0.41	2.06±0.43

Data presented as mean ± SD

Table 11: Mixed two-way ANOVA results for muscle thickness data: time and group effects.

Mixed two-way ANOVA results for muscle thickness data									
Muscle	Lower muscle region			Middle muscle region			Upper muscle region		
	Degrees of freedom Factor; Error	F-ratio	*p* value	Degrees of freedom Factor, Error	F-ratio	*p* value	Degrees of freedom Factor; Error	F-ratio	*p* value
Time effect (within subjects)									
Vastus medialis	2; 34	0.004	0.996	2; 34	0.237	0.872	2; 34	10.773	0.000
Vastus lateralis	2; 34	8.677	0.001	2; 34	20.253	0.000	2; 34	8.058	0.001
Rectus femoris	2; 34	3.479	0.042	2; 34	7.303	0.002	2; 34	16.109	0.000
Vastus intermedius	2; 32	15.503	0.000	2; 32	18.205	0.000	2; 32	9.874	0.000
Group effect (between subjects)									
Vastus medialis	1; 17	0.409	0.531	1; 17	0.496	0.491	1; 17	0.088	0.771
Vastus lateralis	1; 17	4.981	0.039	1; 17	3.348	0.85	1; 17	3.347	0.085
Rectus femoris	1; 17	1.381	0.256	1; 17	0.713	0.410	1; 17	0.326	0.576
Vastus intermedius	1; 16	0.619	0.443	1; 16	0.063	0.805	1; 16	0.220	0.642

Lower muscle region (% of the upper edge of the patella to the anterior superior iliac spine): VM=5%, VL=22%, RF and VI=39%; Middle muscle region: VM=22%, VL=39%, RF and VI=56%; Upper muscle region: VM=39%, VL=56%, RF and VI=73%.

At the middle of the muscle belly (VM: 22%; VL: 39%; RF and VI: 56%), MT was largest in the VM in all groups, followed by the VL and the RF. The VI showed the smallest MT of all studied muscles, irrespectively of the group (Table 10).

Table 11 summarises the results of mixed two-way ANOVA analysis. Strength training resulted in an increase of MT at all muscles and sites excepting the VM. No differences in MT were observed between GCon and GEcc for any of the four muscles and muscle sites. Pairwise comparisons revealed that MT increased from pre-training to after 6 weeks and 15 weeks of strength training for all muscles and sites, excepting for the lower site of the VI and RF and for the upper site of the VM. For VI and RF, MT values increased from pre-training to week 6 (p<0.001) and week 15 (p<0.01), but decreased from week 6 to week 15 (p=0.049 for both muscle sites). For the VM, MT decreased between pre-training and week 15 (p<0.01) and between week 6 and week 15 (p<0.001).

For MT measurements belonging to the lower site of the VL, a significant interaction between time, type of training and leg was found [$F_{(2,34)}$=3.723; p=0.035], which seems to result from differences between the two legs in the response to the Conc training.

Echo-intensity

Tables 12 and 13 present the data for EI. The lowest EI values were registered in the RF and VI, whereas the VL and VM showed higher EI values (Table 12). Changes in EI values with strength training differed between the muscles. Mixed two-way ANOVA results revealed no differences in EI values across time for the VM and RF muscles (Table 13). In the VL and VI muscles, EI values changed significantly with strength training, although in the VL this was true only for the lower and middle portions of the muscle (Table 13). In the lower portion of the VL, pairwise comparisons showed a significant increase in EI values from baseline to week 6 (p=0.028) and week 15 (p=0.007). For the middle portion of the VL, a significant decrease in EI value between baseline and both week 6 and week 15 (respectively, p=0.039 and p=0.001). For the VI muscle, EI values lowered significantly from baseline to week 6 (p<0.001) and week 15 (p<0.001) in the lower portion of the muscle. For the upper portion of the VI, EI values changes were similar to those found for the lower portion, with significant decreases in EI values from baseline to week 6 (p<0.001) and week 15 (p<0.001). In the middle region of the VI, changes in EI values during the strength training weeks were less clear, decreasing at the end of the first 6 weeks compared to baseline (p<0.001) and rising significantly afterwards when compared to baseline (p=0.039) and week 6 (p<0.001).

Table 12: Data of echo-intensity for each four heads of quadriceps.

Echo-intensity (a.u.)							
		Baseline		6 weeks		15 weeks	
Group	Muscle	Right side	Left side	Right side	Left side	Right side	Left side
Control	Vastus medials	81.1±3.40	71.32±5.36	-	-	67.25±5.90	68.95±13.84
	Vastus lateralis	84.9±6.27	81.0±6.52	-	-	79.04±11.94	80.78±14.32
	Rectus femoris	66.21±5.19	61.91±5.35	-	-	60.38±9.68	55.21±6.29
	Vastus intermedius	59.28±17.62	61.65±12.43	-	-	43.17±9.71	60.78±8.90
Concentric training	Vastus medials	76.21±9.89	75.71±11.38	81.44±10.67	73.72±8.31	75.41±10.44	72.52±8.92
	Vastus lateralis	75.07±10.97	80.15±14.54	82.25±12.89	83.11±12.12	76.63±14.58	83.95±13.12
	Rectus femoris	58.84±9.93	61.28±11.71	58.36±17.8	62.2±19.07	58.98±13.38	58.68±12.83
	Vastus intermedius	62.43±21.01	59.74±11.78	50.94±12.73	50.71±12.13	49.2±12.91	46.32±12.33
Eccentric training	Vastus medials	76.86±14.06	76.05±11.41	75.51±11.64	72.77±10.45	70.09±9.19	71.35±11.94
	Vastus lateralis	80.3±15.89	82.3±16.5	79.53±12.19	76.57±14.19	79.44±12.64	84.32±11.25
	Rectus femoris	59.6±13.93	58.51±11.37	60.3±14.47	60.12±13.49	57.74±11.71	56.62±16.6
	Vastus intermedius	71.71±19.06	68.22±16.5	50.88±14.55	47.61±11.52	53.15±16.6	50.61±13.71

Data presented as mean ± SD

Table 13: Mixed two-way ANOVA results for muscle echo-intensity data: time and group effects

Mixed two-way ANOVA results for muscle echo-intensity data									
Muscle	Lower muscle region			Middle muscle region			Upper muscle region		
	Degrees of freedom Factor; Error	F-ratio	*p* value	Degrees of freedom Factor, Error	F-ratio	*p* value	Degrees of freedom Factor; Error	F-ratio	*p* value
Time effect (within subjects)									
Vastus medialis	2; 32	1.008	0.376	2; 32	1.492	0.240	2; 32	2.136	0.135
Vastus lateralis	2; 32	5.648	0.008	2; 32	6.655	0.004	2; 32	1.075	0.353
Rectus femoris	2; 32	2.478	0.100	2; 32	0.140	0.870	2; 32	2.029	0.148
Vastus intermedius	2; 32	72.409	0.000	2; 32	29.960	0.000	2; 32	27.040	0.000
Group effect (between subjects)									
Vastus medialis	1; 16	1.453	0.246	1; 16	1.984	0.008	1; 16	0.264	0.614
Vastus lateralis	1; 16	0.061	0.808	1; 16	0.104	0.751	1; 16	0.532	0.476
Rectus femoris	1; 16	0.144	0.709	1; 16	0.002	0.000	1; 16	0.008	0.928
Vastus intermedius	1; 16	1.109	0.308	1; 16	0.763	0.395	1; 16	0.497	0.491

Lower muscle region (% of the upper edge of the patella to the anterior superior iliac spine): VM=5%, VL=22%, RF and VI=39%; Middle muscle region: VM=22%, VL=39%, RF and VI=56%; Upper muscle region: VM=39%, VL=56%, RF and VI=73%.

No relationship existed between MT and EI for the VM, VL, and RF muscles both before and after strength training (Figure 20). A significant positive relationship between MT and EI was found in the VI at baseline and at week 15 (Figures 21 and 22).

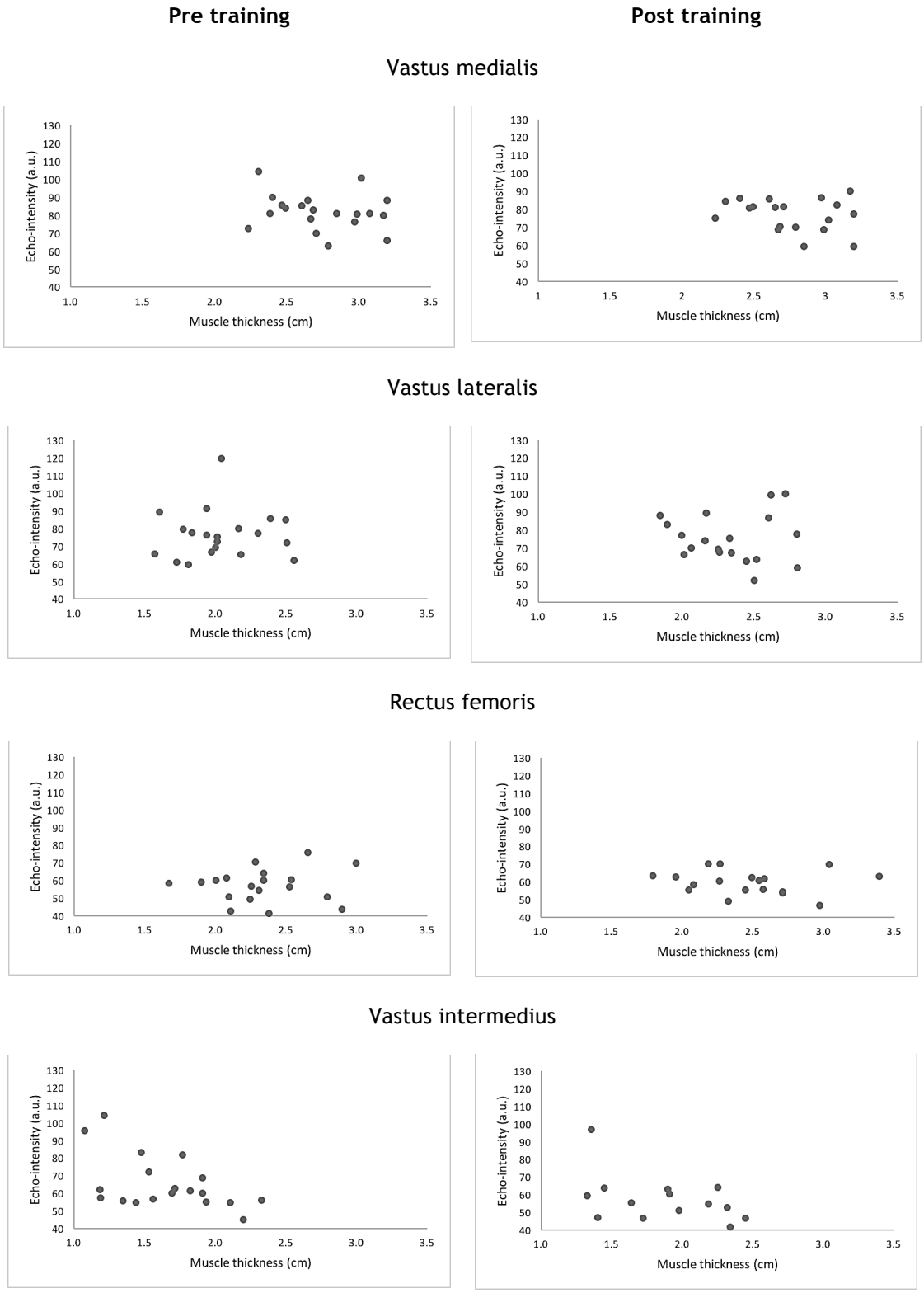


Figure 20: Scatterplots between MT and EI data.

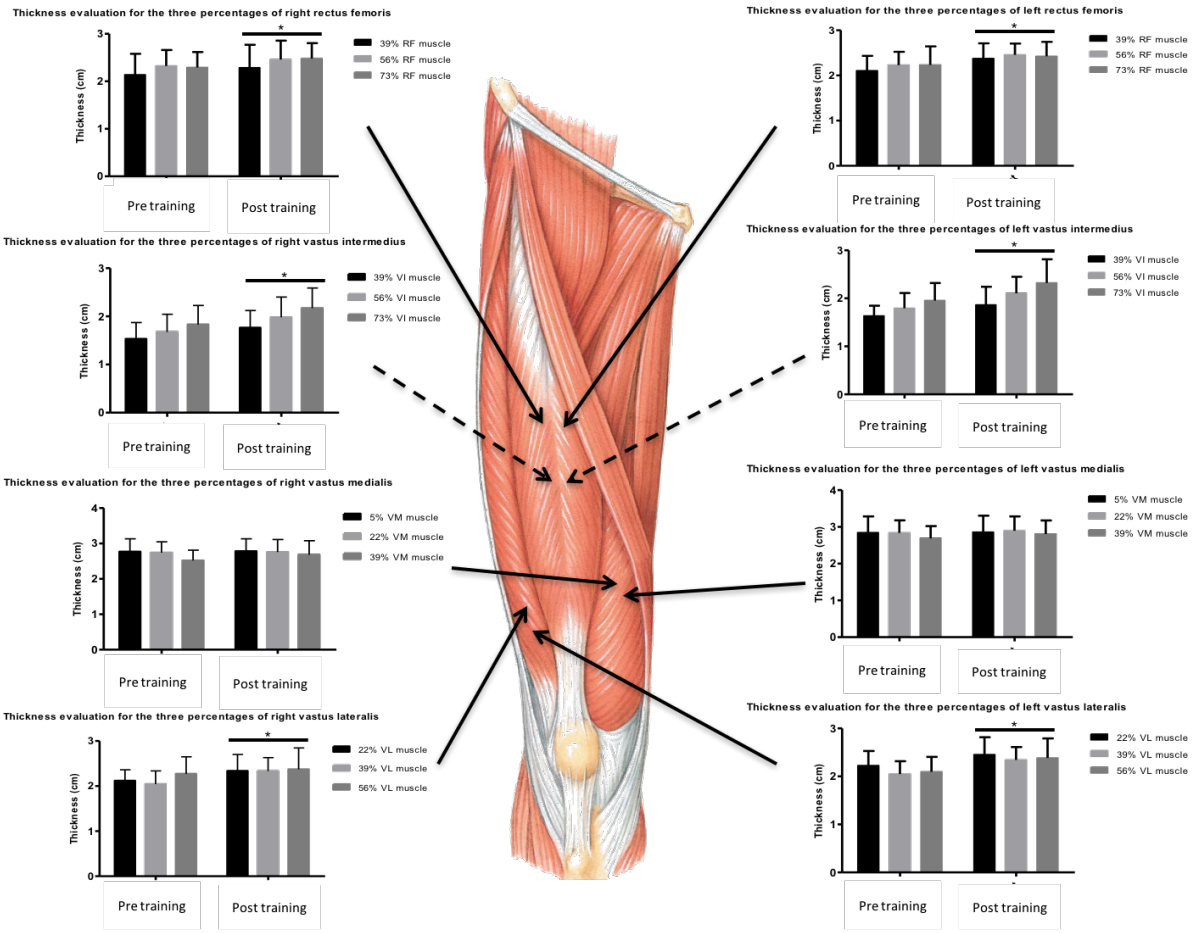


Figure 21: Data for muscle thickness for each evaluated muscle and site at the beginning and end of the study. Values are from the two strength-training groups pooled together. *Significantly different from pre-training; $p<0.05$.

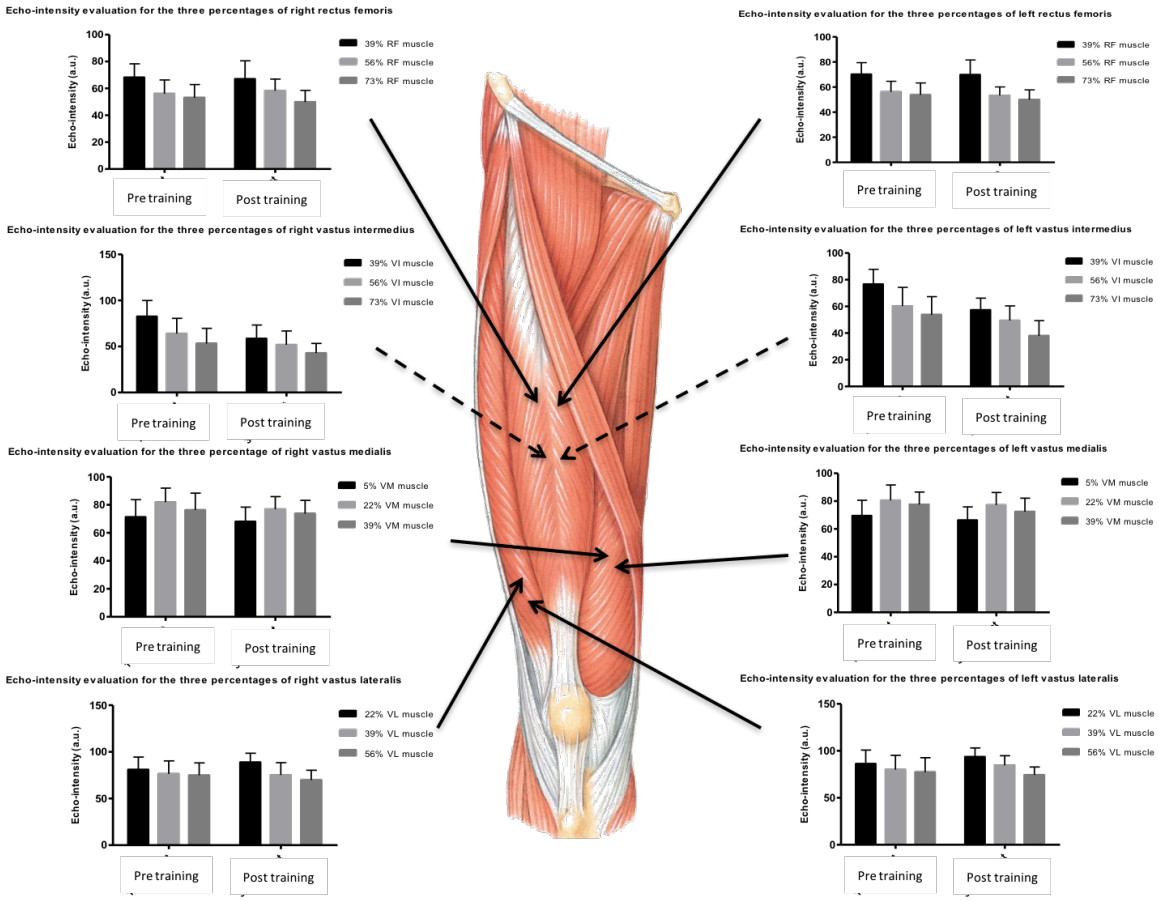


Figure 22: Data for muscle echo-intensity for each evaluated muscle and site at the beginning and end of the study. Values are from the two strength-training groups pooled together.

3.1.3 Discussion

The results of this study showed that 15 weeks of strength training significantly increased knee extension maximal isometric torque, which was accompanied by changes in MT and EI in the muscles composing the quadriceps femoris. However, considerable variation existed in MT and EI changes in response to strength training between the knee extensor muscles and between different regions of the same muscle.

Higher values for MT and EI were observed in the VM and VL muscles for both right and left sides, which is in line with the literature. Nishihara et al. (2014) reported mean MT values for the RF (~2.16 cm) and the VI (~1.61 cm) muscles that are similar to those found in this study. Strasser et al. (2013) also reports MT values for the knee extensors that are comparable to the values we found for each of the heads of the quadriceps femoris, although slightly lower, and that ranged between a minimum of 1.3 cm in the VI and a maximum of 3 cm in the VM. More recently, Herrick et al. (2017) also reported a total thickness for the RF and VI muscles in golf players that is similar to our data

(Herrick et al., 2017). Regarding EI, Nielsen et al. (2006) showed that the VL muscle has a higher grayscale intensity when compared to the other knee extensor muscles, caused by a greater number of non-contractile components (Nielsen et al., 2006). However, Strasser et al. (2013) report mean EI values that were higher in the RF muscle (102 a.u.), followed by the VL (96 a.u.), the VM (94 a.u.), and VI (78 a.u.) (Strasser et al., 2013). These EI values are slightly higher than those we have found in the present study, a difference, which may be explained by differences in the protocol or in the machine. Varanoske et al. (2017) assessed EI in panoramic transverse images of the VL and concluded that EI values were relatively homegenous in different regions of the muscle, with mean EI of around 58 a.u. (Varanoske et al., 2017). Such mean EI value is lower than the one we found but the difference in this case might rely on differences between and transverse and longitudinal plane images. However, our data also show that the VL together with the VM display higher EI compared to the RF and the VI. Therefore, there is agreement between our EI data and the data found in previous studies.

Variations in echo intensity values between muscle groups may be the result of differences in the amount of connective or fibrous tissue in the muscle, fiber type distribution, intramuscular triglyceride arrangement, and architectural features of fascicles and their orientation within separate muscles (Varanoske et al., 2017). On the other hand, the inconsistent depths of the VL captured in each portion and is asymmetrical form; the anteromedial portion of the muscle is thicker than the posterolateral portion can help explaining its higher EI (Varanoske et al., 2017).

Strength training showed a different impact on MT depending on the muscle. Increases in MT with strength training were more clearly seen in the VL and VI. In the VM, strength training did not alter MT values. Our data also show that increases in MT caused by strength training occurred mainly during the first 6 weeks and then tended to level off or even regress. This is contrary to the well established idea that muscle hypertrophy is linearly related with the duration of strength training after an initial period of few weeks. However, the design of the strength training protocol, with the progression based on increasing the velocity of the isokinetic contractions, which at least for the Conc contractions is accompanied by decreased force production, might have contributed to such findings.

The effect of strength training on EI values showed marked differences between the individual knee extensor muscles. The most consistent impact of strength training on EI

values was seen in the VI, where a decrease in EI was found after strength training. In the VL, strength training was also associated with changes in EI values but these changes were in opposite directions in the middle and lower portions of this muscle. In addition, the VI was the only muscle of the quadriceps femoris showing a relationship between MT and EI, in this case a negative one. The reason behind such differences between the quadriceps femoris' heads is not totally understood but might be related to anatomical features of each muscle, in particular the complex spatial arrangement of the muscle fascicles as they course from the proximal to the distal fasciae and the way such large muscles converge distally to form the thick quadriceps tendon, which inserts into the superior border of the patella (Andrikoula et al., 2006; Farahmand et al., 1998; Pasta et al., 2010). In the VM, unchanged EI values with strength training might be related with the fact that changes in MT with strength training were not consistent or significant in this muscle. This explanation for unchanged EI values does not apply to the VL. In this case, the fact that EI values after strength training remained unchanged whereas MT increased may signify that the relative proportion of muscle tissue and connective tissue within the muscle belly does not change with strength training in young participants.

This study showed that both Conc and Ecc strength training produced similar changes in EI and MT. Previously, Batista et al. (2016) had already showed that Conc and Ecc strength training produced similar changes in muscle architecture and strength in healthy elderly subjects (Baptista et al., 2016). Also, Blazevich et al. (2007) has shown that changes in MT produced by Conc and Ecc training were comparable (Blazevich et al., 2007).

3.1.4 Limitations

The main limitation in this study was the inability to use the data from the Control group due to severe drop out. The fact that the muscle sites were selected for scanning based on published data and not on an electromyographic evaluation of the relative participation of each muscle during the training program could have also affected our ability to determine the best muscle sites for data collection.

3.1.5 Summary

A 15-week strength training program produced a significant increase of MT in different regions of the quadriceps femoris but generally failed in changing its EI values. The main exception was the VI, where strength training produced a significant decrease in EI

values. Further studies are needed to explain the variation in EI values and their responsiveness to strength training across the quadriceps femoris.

3.1.6 References

Alqahtani, M. (2010). *Quantitative Ultrasound Image Analysis of the Gastrocnemius Muscle for Injury Evaluation*. Cardiff University.

Andrikoula, S., Tokis, A., Vasiliadis, H., & Georgoulis, A. (2006). The extensor mechanism of the knee joint: An anatomical study. *Knee Surgery, Sports Traumatology, Arthroscopy*, *14*(3), 214–220.

Asakawa, D., Pappas, G., Delp, S., & Drace, J. (2000). Architecture of the biceps brachii characterized with ultrasound and MRI. In *24th annual meeting of the American Society of Biomechanics* (pp. 1–2). Chicago.

Baptista, R., Onzi, E., Goulart, N., Santos, L., Makarewicz, G., & Vaz, M. (2016). Effects of Concentric Versus Eccentric Strength Training on the Elderly's Knee Extensor Structure and Function. *Journal of Exercise Physiology Online*, *19*(3), 120–133.

Blazevich, A., Cannavan, D., Coleman, D., & Horne, S. (2007). Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles, 1565–1575.

Cadore, E. (2012). *Strength and endurance training in elderly men. (Tese de Doutoramento)*. Public University of Navarre.

Cadore, E., Izquierdo, M., Conceição, M., Radaelli, R., Pinto, R., Baroni, B., … Kruel, L. (2012). Echo intensity is associated with skeletal muscle power and cardiovascular performance in elderly men. *Experimental Gerontology*, *47*(6), 473–8.

Caresio, C., Molinari, F., Emanuel, G., & Minetto, M. (2015). Muscle echo intensity: Reliability and conditioning factors. *Clinical Physiology and Functional Imaging*, *September*(5), 1–11.

Farahmand, F., Senavongse, W., & Amis, A. (1998). Quantitative Study of the Quadriceps Muscles and Trochlear Groove Geometry Related to Instability of the Patellofemoral Joint. *J Orthop Res*, *16*(1), 136-43.

Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., … Ichihashi, N. (2012). Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *European Journal of Applied Physiology*, *112*(4), 1519–25.

Gabrielle, K., Nazarian, L., & Walz, D. (2013). US of the Elbow: Indications, Technique, Normal Anatomy, and Pathologic Conditions. *Radiographics*, *33*(July-August), 125–147.

Herrick, I., Brown, S., Agyapong-Badu, S., Warner, M., Ewings, S., Samuel, D., & Stokes, M. (2017). Anterior Thigh Tissue Thickness Measured Using Ultrasound Imaging in Older Recreational Female Golfers and Sedentary Controls. *Geriatrics*, *2*(1), 10.

Maeda, K., Utsu, M., & Kihaile, P. (1998). Quantification of sonographic echogenicity with graylevel histogram width : a clinical tissue characterization. *Ultrasound in Medicine & Biology*, *24*(2), 225–234.

Nielsen, P., Jensen, B., Darvann, T., Jørgensen, K., & Bakke, M. (2006). Quantitative ultrasound tissue characterization in shoulder and thigh muscles--a new approach. *BMC Musculoskeletal Disorders*, *7*, 2.

Norrbrand, L. (2010). *Acute and Early Chronic Responses To Resistance Exercise Using Flywheel or Weights*. Mid Sweden University.

O'Neil, J. (2008). *Musculoskeletal Ultrasound - Anatomy and Technique*. (Springer, Ed.) (1st ed.).

Pasta, G., Nanni, G., Molini, L., & Bianchi, S. (2010). Sonography of the quadriceps muscle: Examination technique, normal anatomy, and traumatic lesions. *Journal of Ultrasound*, *13*(2), 76–84.

Pillen, S. (2010). Skeletal muscle ultrasound. *European Journal Translational Myology*, *1*(4), 145–155.

Pillen, S., Tak, R., Zwarts, M., Lammens, M., Verrijp, K., Arts, I., … Verrips, A. (2009). Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound in Medicine & Biology*, *35*(3), 443–6.

Ruas, C., Pinto, R., Lima, C., Costa, P., & Brown, L. (2017). Test-Retest Reliability of Muscle Thickness, Echo-Intensity and Cross Sectional Area of Quadriceps and Hamstrings Muscle Groups Using B-mode Ultrasound. *International Journal of Kinesiology and Sports Science*, *5*(1), 35.

Strasser, E., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–2388.

Trip, J., Pillen, S., Faber, C., Engelen, B., Zwarts, M., & Drost, G. (2009). Muscle ultrasound measurements and functional muscle parameters in non-dystrophic myotonias suggest structural muscle changes. *Neuromuscular Disorders : NMD*, *19*(7), 462–467.

Varanoske, A., Fukuda, D., Boone, C., Beyer, K., Stout, J., & Hoffman, J. (2017). Homogeneity of echo intensity values in transverse ultrasound images. *Muscle & Nerve*, 19–21.

Vlychou, M., & Teh, J. (2008). Ultrasound of muscle. *Current Problems in Diagnostic Radiology*, *37*(5), 219–230.

Walker, F., Cartwright, M., Wiesler, E., & Caress, J. (2004). Ultrasound of nerve and muscle. *Clinical Neurophysiology*, *115*(3), 495–507.

Wilhelm, E., Rech, A., Minozzo, F., Radaelli, R., Botton, C., & Pinto, R. (2014). Relationship between quadriceps femoris echo intensity, muscle power, and functional capacity of older men. *Age*, *4*(Fev 11), 1–6.

3.2 The effect of strength training on vastus lateralis' stiffness: an ultrasound quasi-static elastography study

The skeletal muscle is a dynamic tissue with a notable capacity to adjust its physiological and mechanical characteristics to the patterns of stretching and contraction they have to perform (Chan et al., 2012; Foure et al., 2012). Muscle stiffness or hardness, which may be simply defined as the resistance offered by muscles to compression, is a mechanical property that adapts in response to muscle use (Mitsuyoshi et al, 2012). Unlike other tissues, skeletal muscle stiffness varies dynamically with muscle length changes and contraction status (Akagi & Kusama, 2015; Gennisson et al., 2005; Ishikawa et al., 2016; Nordez et al., 2009; Nordez et al., 2006). In addition to such transient changes, pathological modifications of the muscle tissue as a result of MSK disease, often affect its stiffness (Botar-Jid et al., 2010, 2012; Park & Kwon, 2012; Sikdar et al., 2009). Repeated muscle use, including resistance training is also known to induce significant, albeit reversible, changes in muscle stiffness (Murayama et al., 2000; Ocarino et al., 2008; Tieleman et al., 2012; Yanagisawa et al., 2011).

Although muscle stiffness may be assessed subjectively by palpation or quantitatively using hardmeter devices, it may also be evaluated using imaging techniques, namely by ultrasound QSE (Brandenburg et al., 2014; Cortes et al., 2015; Drakonaki et al., 2012; Eby et al., 2015). QSE is nowadays implemented in many commercial US imaging machines, allowing real-time visualization of the amount of muscle tissue strain caused by repetitive light compressions applied directly onto the muscles with the US probe. While QSE was initially developed to enable an easier identification of abnormal structures on the basis of their higher stiffness relatively to the surrounding healthy tissue, it was soon applied for measuring muscle stiffness in active muscles (Botar-Jid et al., 2010). With QSE, tissue strain magnitude caused by the compressive stresses is depicted as a colour-coded strain map (also termed "elastogram"), overlaid to the B-mode image. Also, semi-quantitative measurements of tissue stiffness may be gathered from elastograms using colour pixels counting (Drakonaki et al., 2012; Ophir et al., 2002). In relaxed skeletal muscles, elastograms typically display a sparse colour pattern, characterized by scattered harder (blue, bluish colours) and softer (red, reddish colours) regions (Drakonaki et al., 2012; Vasilescu et al., 2010). QSE has been successfully used to study skeletal muscle stiffness associated with MSK disorders (Botar-Jid et al., 2010, 2012; Drakonaki & Allen, 2010; Vasilescu et al., 2010), including muscle spasticity (Vasilescu et al., 2010), myofascial trigger points (Sikdar et al., 2009),

muscle contractures associated with joint dysfunction (Chan et al., 2012), and congenital muscle dystrophy (Drakonaki & Allen, 2010).

Together with its widespread use in the clinic, US elastography has also been used during normal skeletal muscle contractile activity (Ishikawa et al., 2015, 2016; Yanagisawa et al., 2011; Yanagisawa, 2015). Compared to other techniques, US elastography is advantageous since muscle stiffness can be assessed dynamically and in real-time in separate regions of muscles (e.g. the muscle belly and the tendon), in superficial and deep muscles, and in complex multifunctional muscle groups, like in the neck (Ishikawa et al., 2016) and in the shoulder girdle (Ishikawa et al., 2015; Muraki et al., 2015). In the shoulder, US strain elastography allowed to monitor the state of contraction of single muscles within a muscle group (Ishikawa et al., 2015; Muraki et al., 2013; Muraki et al., 2015).

Only few studies have employed QSE to investigate changes in skeletal muscle stiffness in response to strength or endurance training (Botar-Jid et al., 2012; Lalitha et al., 2011). To our knowledge, only one study has used US elastography (shear wave elastography) to investigate the impact of strength training (six weeks) for the elbow extensors on triceps brachii's stiffness, and could not find any effect of training on this property (Akagi et al., 2016). Therefore, the potential of QSE to reveal changes in skeletal muscle stiffness after strength training is not fully known.

Therefore, the aim of this study is to evaluate changes in muscle stiffness caused by Conc and Ecc strength training on knee extensors using QSE.

3.2.1 Materials and Methods

The details regarding the participants, strength training protocol and muscle strength testing were described in the previous study. Here, we will only describe the procedures employed for collecting the QSE data and the statistical analysis.

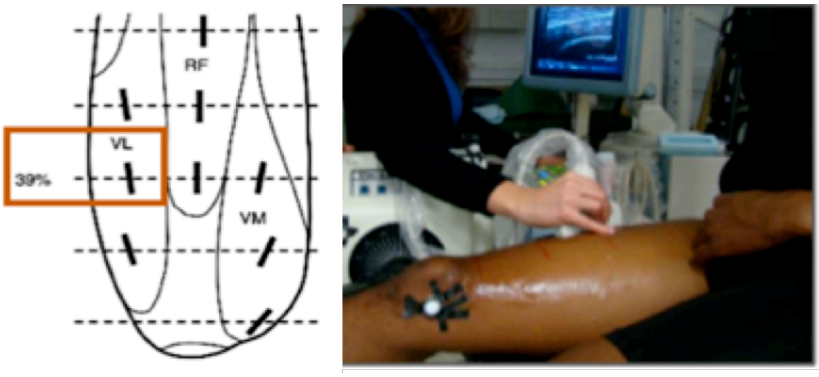


Figure 23: Diagram showing the location for ultrasound scanning (left). Picture of one participant sat on the dynamometer during ultrasound scanning (right).

Ultrasound scanning

Ultrasound scans were collected from the VL bilaterally at a region located at 39% of the distance between the upper edge of the patella and the anterior superior iliac spine (Figure 23) at baseline and after strength training using a commercial US system (HITACHI EUB 7500, HITACHI Medical Corporation, Tokyo, Japan), equipped with a 7-12 MHz linear probe. The US scans were acquired with participants seated on the isokinetic dynamometer, with 10° of knee flexion, and muscles fully relaxed. The probe was placed aligned with the muscle fascicles (longitudinal scan). An experienced certified MSK ultrasound sonographer was responsible for collecting and analysing US data. To dismiss the possibility of circadian effects, the same person attended the laboratory for US data collection at approximately the same time of day before and after strength training.

Ultrasound settings were adjusted and optimized individually during the first session. The settings were then registered and implemented again during the second visit. The US gain was set at 25% of its range, dynamic range was maintained at 70 dB, time compensation was kept at neutral position, and depth was fixed at 65 mm for all scans. For strain elastography acquisition, light compressions were applied with the US probe at a frequency 3-4 Hz using the feedback provided by the manufacturer's software and displayed on the screen. Elastography images were recorded in video sequences and in bitmap format and stored in a computer hard disk. Two images were selected from each video for measurement of muscle stiffness.

Semi-quantitative elastography

Muscle stiffness was given semi-quantitatively by the fraction of red, green, and blue pixels measured within a ROI using a routine written in MATLAB 20.0 software (The MathWorks, Inc., Natick, Massachusetts, USA) (Figure 24). For each image, a rectangular ROI was draw centred on the VL and with a minimum size of 5 x 7 mm. Tissue elasticity

was represented by colour-coding (Botar-Jid et al., 2012). Each pixel within the elastogram is assigned one of 256 specific colours depending on the amplitude of deformation, but three basic colours were used, called encoding RGB (red-green-blue). Colour ranged from red, corresponding to softer tissues, to blue, corresponding to harder tissues responding with less deformation to the applied pressure (Botar-Jid et al., 2012; Ge et al., 2015). The green colour indicates tissues with medium deformation and lying between the red and blue coded tissues (Ge et al., 2015).

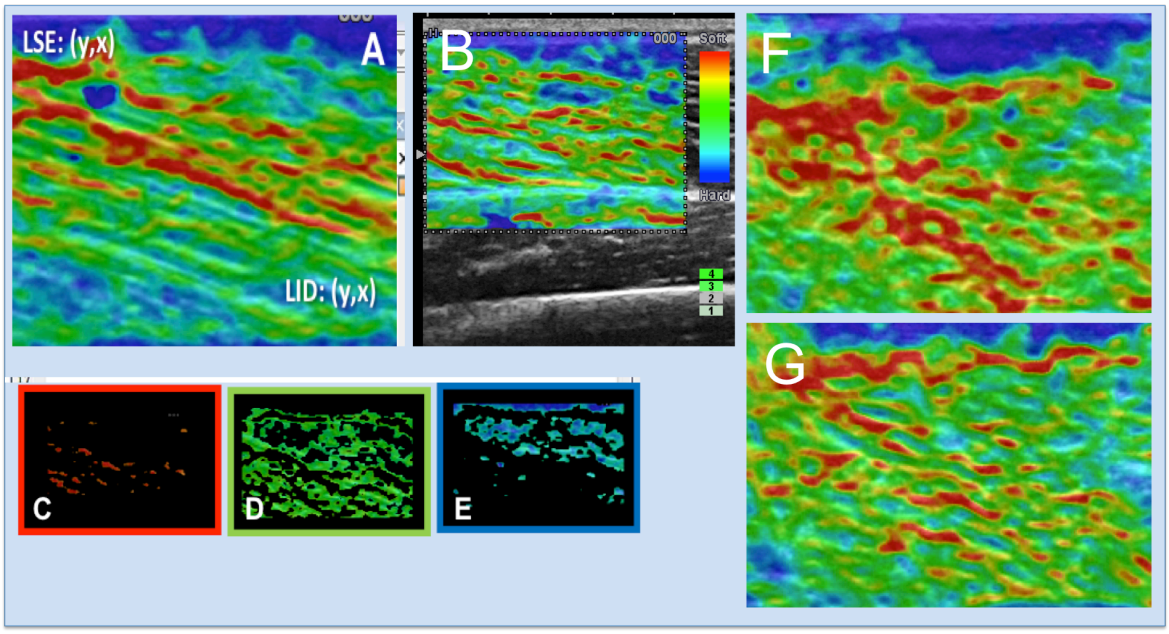


Figure 24: Strain elastography maps and red, green, and blue pixels counting. (A) Elastogram map opened with the MATLAB routine. (C-E) Red, green, and blue pixels from A. (B) Elastogram superimposed to the B-mode image. (F) VL's elastogram at baseline. (G) VL's elastogram after resistance training (visible the decrease in the amount of red pixels).

Statistical analysis

In the control group, measures of knee torque and of VL elastography were collected only from the right leg. Therefore, statistical analysis for the effect of strength training was performed separately for the control group and the two other groups. Pairwise comparisons were performed using t-test (control group). For the other two groups, differences in knee maximal torque and in percentage of colour pixels before and after strength training were tested by mixed two-way analysis of variance (ANOVA) with a between-subjects factor with two levels (GCon and GEcc groups) and two within-subjects' factors, both with two levels (pre-and-post strength training and right and left leg). The sphericity assumption was assessed with Mauchly's test and if necessary the Greenhouse-Geiser corrected significance level was considered. Correlations between

elastography and muscle strength data were studied using linear regression and Pearson correlation coefficient.

Intra-session (intra-observer) reliability for QSE values was assessed using ICC (3,1; method: two-way mixed, consistency) and its 95% interval of confidence. SEM and SDC were also calculated. SEM indicates the precision of the measurement and was calculated based on the ICC and the SD of the mean of the differences between the two measurements (i.e., SEM = SD√1-ICC). The SDC was based on the SEM, using the formula SDC = 1.96*√2*SEM.

Data is reported as mean ± SD. Threshold for statistical significance was set at $p<0.05$. All analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package.

3.2.2 Results

Table 14 shows the data for maximal knee extensor torque before and after strength training.

Table 14: Data for maximal isometric torque produced by the knee extensors before and after strength training for right and left limbs.

	Maximal voluntary isometric torque (Nm)			
	Pre-training		Post-training	
Group	Right limb	Left limb	Right limb	Left limb
Control	303.5±30.8		300.0±35.7	
GCon	300.3±55.2	284.1±46.7	377.3±54.6*	374.3±54.6*
GEcc	241.9±35.0	229.2±12.5	328.9±42.8*	328.9±42.8*

Data presented as mean ± SD.
*Significantly different from pre-training ($p<0.001$)

After strength training, maximal knee extensor torque increased significantly in both training groups [$F_{(1,18)}=58.583$; $p<0.001$], but it did not change in the control group ($p=0.47$). The increase in maximal knee extensor torque was similar in the GCon and GEcc groups as well as in the two limbs [pre-post vs. group interaction, $F_{(1,18)}=0.006$; $p=0.938$; pre-post vs. left-right interaction, $F_{(1,18)}=0.149$; $p=0.704$].

Elastogram

Table 15 presents the mean red, green and blue colour pixel fractions within VL's elastograms.

Regardless of group, leg, or time point, VL's elastograms showed lower fraction number of red pixels when compared to that of the green and blue ones. The percentage of red pixels, expressed relatively to the total amount of pixels within each elastogram (Figure 24) varied between approximately 8% to around 16%. The percentage of green and blue pixels was similar, ranging from approximately 26% to 38.7% (Table 15).

Table 15: Data for colour pixels in vastus lateralis' elastograms.

Fraction of colour pixels (a.u.)					
		Pre-training		Post-training	
Group	Colour	Right side	Left side	Right side	Left side
Control	Red	0.156±0.058		0.144±0.065	
	Green	0.297±0.043		0.325±0.079	
	Blue	0.261±0.067		0.333±0.079*	
GCon	Red	0.160±0.050	0.111±0.052	0.082±0.023**	0.088±0,027**
	Green	0.295±0.037	0.319±0,043	0.341±0.039*	0.354±0.041*
	Blue	0.290±0.090	0.354±0.095	0.381±0.076§	0.359±0.063§
GEcc	Red	0.126±0.056	0.131±0.063	0.092±0.020**	0.089±0.021**
	Green	0.311±0.048	0.284±0.036	0.348±0.063*	0.313±0.073*
	Blue	0.344±0.096	0.368±0.010	0,357±0.069§	0.387±0.097§

Data are presented as mean ± SD.
Significantly different from Pre-training: $p<0.05$§; $p<0.01$*; $p<0.001$**.

In the control group, no changes in the fraction number of red and green pixels existed when comparing the VL's elastograms at baseline with those collected at the end of the study. However, this group showed a slight, although significant, increase in the mean amount of the fraction number of blue pixels at the end of the study ($p<0.01$). After strength training, significant changes in the fraction number of colour pixels could be detected in VL's elastograms. Specifically, strength training diminished the fraction number of soft, red pixels [$F_{(1,18)}=25.490$; $p<0.001$], and increased that of the harder green and blue pixels [pre vs. post strength training for green and blue pixels respectively: $F_{(1,18)}=17.179$; $p<0.01$; $F_{(1,18)}=6.522$; $p<0.05$]. These data are compatible with increased muscle stiffness as a result of strength training. Changes in the relative amounts of red, green and blue colour pixels after strength training were similar in GCon and GEcc groups and between the right and left legs.

Before strength training, maximal knee extensor torque was positively correlated with the percentage of red pixels within the VL's elastograms ($r^2=0.43$; $p<0.01$) and negatively correlated with the percentage of blue pixels ($r^2=0.29$; $p<0.05$). After

training, no correlations between maximal knee extensor torque and number of red, green or blue colour pixels within VL's relative strain maps could be found.

Table 16 reports the reliability data regarding colour-mapping measures. Moderate intra-session ICCs were found. For all the three colours, ICC values were around the 0.6 acceptance level.

Table 16: Data of colour mapping for vastus lateralis and intra-class correlation coefficient (n= 8).

Colour mapping for Vastus Lateralis	1st image Mean fraction	ICC Consistency	SEM	SDC
Right Side				
Red colour	0.15±0.07	0.59	0.05	0.13
Blue colour	0.30±0.11	0.77	0.06	0.16
Green colour	0.30±0.05	0.53	0.04	0.11
TOTAL	0.75			
Left Side				
Red colour	0.12±0.07	0.67	0.04	0.12
Blue colour	0.35±0.10	0.68	0.06	0.16
Green colour	0.31±0.05	0.63	0.04	0.10
TOTAL	0.78			

Data presented as mean ± SD
ICC= intra-class correlation coefficient; SEM= Standard error of measurement; SDC= smallest detectable change

SEM values for each of the three colours were similar, ranging between 0.04 and 0.06. SDC values varied between 0.10 (green colour for left thigh) and 0.16 (blue colour for both thighs) (Table 16). Blue colour tended to show higher absolute SEM and SDC values compared to red and green colours. Contrarily, SEM and SDC values for green colour were consistently smaller (Table 16).

Table 17 reports the ICCs for data recorded in the same session (intra-session reliability). Moderate intra-observer ICCs were found. Only the measurements of green pixel fractions presented ICC values under the 0.6 acceptable level.

Table 17: Data of colour mapping for vastus lateralis and inter-class correlation coefficient (n= 7).

GROUP	Colour mapping for vastus lateralis	1st session Mean fraction	2nd session Mean fraction	ICC Consistency
Control	Red colour	0.15±0.05	0.11±0.02	0.44
	Blue colour	0.25±0.08	0.34±0.11	0.75
	Green colour	0.32±0.07	0.33±0.07	0.60

Data presented as mean ± SD; ICC= intra-class correlation coefficient

3.2.3 Discussion

This QSE study demonstrated that VL hardness increases after 15 weeks of strength training and in a similar degree with both Ecc and Conc training and with smaller or larger knee ranges of motion. To the best of our knowledge, this is the first time that increased muscle stiffness as a result of strength training is demonstrated by means of US elastography.

Recently, muscle hardness monitoring has become a relatively accessible way for evaluating acute stiffness tissue changes caused by repeated muscle activity (Hirono et al., 2016). Despite the limitations of US elastography, its feasibility as a means to assess passive muscle tissue stiffness has been demonstrated by several studies (Brandenburg et al., 2014; Hirono et al., 2016; Niitsu et al., 2011).

Colour-coded elastograms of healthy, relaxed skeletal muscles typically display complex and irregular colour patterns in which green and blue colours predominate, meaning that the stiffness of the skeletal muscle tissue is moderate but not strictly homogeneous (Debernard et al., 2011; Drakonaki et al., 2012; Paluch et al., 2016). Our study seems to confirm that VL's hardness is also heterogeneous, with patchy areas of red colour surrounded by areas of predominant green and blue colours in line with Drakonaky et al (2012) who defined the muscle as "an inhomogeneous mosaic of intermediated or increased stiffness with scattered softer and harder areas" (Drakonaki et al., 2012). Presently, it is not known whether there is any information that can be extracted from the colour or grayscale patterns visible on strain US elastography images of skeletal muscles and whether such information could be associated with anatomical or physiological features. However, when viewed longitudinally, the few patchy areas with the same colour observed in colour-coded elastograms of skeletal muscles resemble the spatial arrangement of muscle fascicles, thus suggesting that a relationship between transverse strain behaviour and the skeletal muscle's architecture may indeed exist. Regardless the apparent stochastic character of colour-coded skeletal muscle elastograms, their relative amounts of softer and harder colours change in a systematic way and in the expected direction (i.e., increased amount of harder colour pixels) when skeletal muscles contract or stretch (Vasilescu et al., 2010). Also, muscle tissue changes associated with congenital and traumatic myopathies are discernible with US strain elastography, which strengthens the validity and sensitivity of this imaging technique for assessing skeletal muscle mechanical properties (Botar-Jid et al., 2010, 2012; Drakonaki et al., 2012; Kim et al., 2015; Paluch et al., 2016).

By using strain US elastography changes in muscle stiffness due to repeated contractile activity have been shown to occur (Chino et al., 2012; Hirono et al., 2016; Muraki et al., 2014; Niitsu et al., 2011; Paluch et al., 2016; Yanagisawa et al., 2015). After a series of forceful Conc and Ecc contractions by the elbow flexors, strain US elastography images showed increased strain resistance in biceps brachii in comparison to that of a constant-stiffness reference material placed in between the US probe and the skin (Yanagisawa et al., 2011). Such changes in biceps brachii's colour-coded elastograms were relatively short-lasting and were accompanied by an increase in muscle hardness, as measured with a tissue hardness meter (Yanagisawa et al., 2011).

The increased muscle stiffness occurring after intense muscle contractions has been explained on the basis of increased hydrostatic pressure, raised muscle blood flow, and elevated intramuscular fluid content, particularly within the extracellular space (Sjogaard & Saltin, 1982; Yanagisawa et al., 2004). Other possibility, which might also underlie the changes in muscles fluid content, is the occurrence of small structural changes or injuries, which might trigger an inflammatory response, alter the stiffness of the muscle regions affected, or even lead to increased residual muscle contraction (Nosaka & Newton, 2002; Toledo, 2016; Yanagisawa et al., 2015). However, it is unlikely that raised intramuscular pressure and exercise-induced muscle oedema could explain the changes in VL's relative stiffness that were observed in the present study as a result of strength training. In our study, participants were tested few days after having performed the last training session and they were carefully instructed to keep their knee extensors relaxed during US scanning. Therefore, the increase in relative strain seen in the US elastograms of strength trained VL is likely related with lasting and adaptive changes in the mechanical characteristics of the muscle tissue, specifically with an increase in muscle tissue stiffness.

Increased muscle stiffness as a result of strength training has been demonstrated in several occasions (Bensamoun et al., 2006; Chleboun et al., 1997; Foure et al., 2012; Ocarino et al., 2008). Muscle stiffness, defined as the relationship between the change in muscle length and the amount of stress applied to the muscle, is generally regarded as the strain resistance along the muscles' longitudinal axis or the resistance to muscle elongation and is given by the steepness of the passive or active length-tension curve of the muscle-tendon complex. The compressive force that is applied onto the muscle tissue during strain US elastography strains the underlying tissues in proportion to their stiffness, which is then derived by contrasting the US echoes during compressed and uncompressed conditions (Drakonaki et al., 2012). Changes in joints' resting position and tendon stiffness after intense muscle contractile activity and strength training are a

further indication of increased muscle hardness and stiffness associated with increased muscle use (Taş et al., 2017). Taş et al. (2017) showed that the quadriceps femoris strength was positively correlated with patellar tendon stiffness and thickness (Taş et al., 2017). After two months of resistance training of the elbow flexors, the resting elbow position significantly moved towards increased flexion, while the elbow joint's stiffness, a parameter that informs about the biomechanical properties of the overall structures making up this joint (i.e. joint surfaces, cartilages, joint capsule and ligaments), including the several muscle-tendon complexes crossing the joint, significantly increased (Ocarino et al., 2008). Increased gastrocnemius muscles stiffness was also reported after plyometric training of the human ankle plantar flexor muscles, a kind of training in which cycles of powerful lengthening and shortening muscle contractions are performed, thus resembling the behaviour of ankle plantar flexors during running or jumping (Foure et al., 2012).

There are several possible mechanisms underlying the increase in muscle stiffness seen after strength training. Changes in muscle architecture and muscle hypertrophy might be responsible for increased muscle passive stiffness (Chleboun et al., 1997; Murayama et al., 2012). A significant positive correlation between biceps brachii's and brachial anterior's volumes and elbow joint stiffness has been reported (Chleboun et al., 1997). Nonetheless, differences in muscle stiffness in response to axial compression and to elongation might exist. Muscle hardness is the term that is usually employed to name the palpable resistance of the muscle tissue to an applied pressure and, in this sense, is closer to strain elastography qualitative or semi-quantitative measures than the biomechanical muscle-tendon complex elongation apparent stiffness. However, muscle hardness and muscle elongation stiffness are correlated and, therefore, a rise in muscle hardness occurs when there is an increase in muscle's tension caused by contraction (Leonard et al., 2004; Murayama et al., 2012) or by passive stretch (Murayama et al., 2012).

In the present study, we had different groups undertaking Conc and Ecc strength training. Also, in one limb muscle contractions were performed along a larger movement amplitude of the knee joint (i.e. knee extensors trained in shortened length). However, strength increase was similar in the two strength-training groups and in both limbs, showing that the efficacy of the strength training, in terms of the gain in maximal voluntary isometric strength, was similar irrespectively of the kind of muscle contraction employed (i.e., Conc vs. Ecc) and the amount of active shortening or lengthening (full ROM vs. limited ROM). Likewise, the percentage changes in "softer" and "harder" colours in US elastograms of the VL after strength training was similar

amongst the different training conditions. Previous studies show that the increase in human elbow flexors stiffness is related with the total amount of muscle work during training (Ocarino et al., 2008). Whether such discrepancies between our results and those of others reflect fundamental differences between elbow flexors and the VL or differences in the training and testing protocols between the two studies is unknown at this point.

It is possible that the similar changes in the US elastograms between the different strength training conditions may be associated with the limited precision of QSE measures. Previously, Muraki et al. (2014), and Yanagisawa et al. (2011) showed very high ICC values (around 0.9) for the strain ratio in the supraspinatus and biceps brachii muscles (Muraki et al., 2014; Yanagisawa et al., 2011). However, large variations in ICC values (range 0.6-0.9) for US elastography measures collected from the gastrocnemius muscle evaluation have also been reported (Hirono et al., 2016). Our study showed a moderate (ICC: 0.6-0.7) intra-evaluation reliability for colour mapping values by QSE US, maybe due to the compression operator-dependence (Gennisson et al., 2013; Konofagou et al., 2003; Taljanovic et al., 2015, Lacourpaille et al., 2012; Park & Kwon, 2012).

3.2.4 Limitations

Strain US elastography offers qualitative information of tissue strain behaviour by direct viewing of a grayscale or colour-coded image superimposed on the B-mode image. However, the range of strains is dynamically adjusted depending on the difference between the lowest and the highest strained tissue within the scanned region, thus rendering the elastogram a relative image only. To make the qualitative colour-coded information of QSE a semi-quantitative measure, ratios of relative strains between the ROI and a reference can be employed. The overlying subcutaneous fat can be used as reference, but it is not possible to guarantee that the subcutaneous fat properties remain unchanged after the training. Moreover, the thickness of the subcutaneous fat may be too small to allow selecting a ROI. This limitation is particularly relevant when studying young and very active subjects, as in this study, who have a very low percentage of body fat. As an alternative, a material of homogeneous mechanical properties might be used for reference (Drakonaki et al., 2012; Yanagisawa et al., 2011). However, our elastographic measures were not normalized to a reference. Therefore, we should emphasize that there is a small chance that the increase in the area fraction occupied by the harder green and blue colours that was noted in our study after strength training might not reflect heightened muscle hardness. Indeed, structural changes could have altered the relative hardness of the different tissues that are

scanned and thus modified the colour distribution within the elastogram. However, increased fraction of "harder" colour in the VL elastograms would have happened only if some tissue harder than the muscle prior training had turned softer by the end of the study, which is unlikely. Another limitation of this study relies on the operator-dependence of QSE. Although this limitation cannot be completely avoided, it was minimised by having the same operator conducting every US scan and by carefully selecting images for analysis obtained when compression frequency stood in the range 3-4 Hz.

3.2.5 Summary

This study offers the first demonstration that after 15 weeks of strength training, there is an increase in the stiffness of the relaxed VL that can be measured with strain US elastography. However, this technique only provides semi-quantitative measures and in the future, it would be important to confirm the results of our study with quantitative US elastography methods.

3.2.6 References

Akagi, R., & Kusama, S. (2015). Comparison Between Neck and Shoulder Stiffness Determined by Shear Wave Ultrasound Elastography and a Muscle Hardness Meter. *Ultrasound in Medicine & Biology*, *41*(8), 2266–71.

Akagi, R., Shikiba, T., Tanaka, J., & Takahashi, H. (2016). A Six-Week Resistance Training Program Does Not Change Shear Modulus of the Triceps Brachii. *Journal of Applied Biomechanics*, *32*(4), 373–378.

Bensamoun, S., Stevens, L., Fleury, M., Bellon, G., Goubel, F., & Ho Ba Tho, M. (2006). Macroscopic-microscopic characterization of the passive mechanical properties in rat soleus muscle. *Journal of Biomechanics*, *39*(3), 568–578.

Botar-Jid, C., Damian, L., Dudea, S., Vasilescu, D., Rednic, S., & Badea, R. (2010). The contribution of ultrasonography and sonoelastography in assessment of myositis. *Medical Ultrasonography*, *12*(2), 120–126.

Botar-Jid, C., Vasilescu, D., Damian, L., Dumitriu, D., Ciurea, A., & Dudea, S. (2012). Musculoskeletal sonoelastography. Pictorial essay. *Medical Ultrasonography*, *14*(3), 239–245.

Brandenburg, J., Eby, S., Song, P., Zhao, H., Brault, J., Chen, S., & An, K. (2014). Ultrasound elastography: The new frontier in direct measurement of muscle stiffness. *Archives of Physical Medicine and Rehabilitation*, *95*(11), 2207–2219.

Chan, S., Fung, P., Ng, N., Ngan, T., Chong, M., Tang, C., … Zheng, Y. (2012). Dynamic changes of elasticity, cross-sectional area, and fat infiltration of multifidus at different postures in men with chronic low back pain. *Spine Journal*, *12*(5), 381–388.

Chino, K., Akagi, R., Dohi, M., Fukashiro, S., & Takahashi, H. (2012). Reliability and Validity of Quantifying Absolute Muscle Hardness Using Ultrasound Elastography, *7*(9), 5–9.

Chleboun, G., Howell, J., Conatser, R., & Giesey, J. (1997). The relationship between elbow flexor volume and angular stiffness at the elbow. *Clinical Biomechanics*, *12*(6), 383–392.

Cortes, D., Suydam, S., Silbernagel, K., Buchanan, T., & Elliott, D. (2015). Continuous Shear Wave Elastography: A New Method to Measure Viscoelastic Properties of Tendons in Vivo. *Ultrasound in Medicine & Biology*, *41*(6), 1518–1529.

Debernard, L., Robert, L., Charleux, F., & Bensamoun, S. (2011). Characterization of muscle architecture in children and adults using magnetic resonance elastography and ultrasound techniques. *Journal of Biomechanics*, *44*(3), 397–401.

Drakonaki, E., Allen, G., & Wilson, D. (2012). Ultrasound elastography for musculoskeletal applications. *The British Journal of Radiology*, *85*(1019), 1435–45.

Drakonaki, E., & Allen, G. (2010). Magnetic resonance imaging, ultrasound and real-time ultrasound elastography of the thigh muscles in congenital muscle dystrophy. *Skeletal Radiology*, 391–396.

Eby, S., Cloud, B., Brandenburg, J., Giambini, H., Song, P., Chen, S., … An, K. (2015). Shear wave elastography of passive skeletal muscle stiffness: Influences of sex and age throughout adulthood. *Clinical Biomechanics*, *30*(1), 22–27.

Foure, A., Nordez, A., & Cornu, C. (2012). Effects of plyometric training on passive stiffness of gastrocnemii muscles and achilles tendon. *European Journal of Applied Physiology*, *112*(8), 2849–2857.

Ge, L., Shi, B., Song, Y., Li, Y., Wang, S., & Wang, X. (2015). Clinical value of real-time elastography quantitative parameters in evaluating the stage of liver fibrosis and cirrhosis. *Experimental and Therapeutic Medicine*, *10*(3), 983–990.

Gennisson, J., Deffieux, T., Fink, M., & Tanter, M. (2013). Ultrasound elastography: principles and techniques. *Diagnostic and Interventional Imaging*, *94*(5), 487–95.

Gennisson, J., Cornu, C., Catheline, S., Fink, M., & Portero, P. (2005). Human muscle hardness assessment during incremental isometric contraction using transient elastography. *Journal*

of Biomechanics, *38*(7), 1543–1550.

Hirono, J., Mukai, N., Takayanagi, S., & Miyakawa, S. (2016). Changes in the hardness of the gastrocnemius muscle during a Kendo training camp as determined using ultrasound real-time tissue elastography. *The Journal of Physical Fitness and Sports Medicine*, *5*(3), 239–245.

Ishikawa, H., Muraki, T., Morise, S., Sekiguchi, Y., Yamamoto, N., Itoi, E., & Izumi, S. (2016). Changes in stiffness of the dorsal scapular muscles before and after computer work: a comparison between individuals with and without neck and shoulder complaints. *European Journal of Applied Physiology*.

Ishikawa, H., Muraki, T., Sekiguchi, Y., Ishijima, T., Morise, S., Yamamoto, N., … Izumi, S. (2015). Noninvasive assessment of the activity of the shoulder girdle muscles using ultrasound real-time tissue elastography. *Journal of Electromyography and Kinesiology*, *25*(5), 723–730.

Kim, S., Park, H., & Lee, S. (2015). Usefulness of strain elastography of the musculoskeletal system. *Ultrasonography*, *35*(April), 104–109.

Konofagou, E., Ophir, J., & Krouskop, T. (2003). Elastography: From Theory To Clinical Applications. In *Summer Bioengineering Conference* (Vol. June, pp. 367–368). Florida.

Lacourpaille, L., Hug, F., Bouillard, K., Hogrel, J., & Nordez, A. (2012). Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. *Physiological Measurement*, *33*(3), N19-28.

Lalitha, P., Reddy, M., & Reddy, K. (2011). Musculoskeletal Applications of Elastography : a Pictorial Essay of Our Initial Experience. *Korean Journal of Radiology*, *12*(3), 365–375.

Leonard, C., Brown, J., Price, T., Queen, S., & Mikhailenok, E. (2004). Comparison of surface electromyography and myotonometric measurements during voluntary isometric contractions. *Journal of Electromyography and Kinesiology*, *14*(6), 709–714.

Muraki, S., Fukumoto, K., & Fukuda, O. (2013). Prediction of the muscle strength by the muscle thickness and hardness using ultrasound muscle hardness meter. *SpringerPlus*, *2*(457), 1–7.

Muraki, T., Ishikawa, H., & Morise, S. (2014). Ultrasound elastography – based assessment of the elasticity of the supraspinatus muscle and tendon during muscle contraction. *Journal of Shoulder and Elbow Surgery*, 1–7.

Muraki, T., Ishikawa, H., Morise, S., Yamamoto, N., Sano, H., Itoi, E., & Izumi, S. ichi. (2015). Ultrasound elastography-based assessment of the elasticity of the supraspinatus muscle and tendon during muscle contraction. *Journal of Shoulder and Elbow Surgery*, *24*(1), 120–126.

Murayama, M., Nosaka, K., Yoneda, T., & Minamitani, K. (2000). Changes in hardness of the human elbow flexor muscles after eccentric exercise. *European Journal of Applied Physiology*, *82*(5–6), 361–367.

Murayama, M., Watanabe, K., Kato, R., Uchiyama, T., & Yoneda, T. (2012). Association of muscle hardness with muscle tension dynamics: A physiological property. *European Journal of Applied Physiology*, *112*(1), 105–112.

Niitsu, M., Michizaki, A., Endo, A., Takei, H., & Yanagisawa, O. (2011). Muscle hardness measurement by using ultrasound elastography: a feasibility study. *Acta Radiologica (Stockholm, Sweden : 1987)*, *52*(1), 99–105.

Nordez, A., Cornu, C., & McNair, P. (2006). Acute effects of static stretching on passive stiffness of the hamstring muscles calculated using different mathematical models. *Clinical Biomechanics*, *21*(7), 755–760.

Nordez, A., Guével, A., Casari, P., Catheline, S., & Cornu, C. (2009). Assessment of muscle hardness changes induced by a submaximal fatiguing isometric contraction. *Journal of Electromyography and Kinesiology*, *19*(3), 484–491.

Nosaka, K., & Newton, M. (2002). Concentric or eccentric training effect on eccentric exercise-induced muscle damage. *Medicine and Science in Sports and Exercise*, *34*(1), 63–69.

Ocarino, J., Fonseca, S., Silva, P., Mancini, M., & Gonçalves, G. (2008). Alterations of stiffness and resting position of the elbow joint following flexors resistance training. *Manual Therapy*, *13*(5), 411–418.

Ophir, J., Alam, S., Garra, B., Kallel, F., Konofagou, E., Krouskop, T., … Varghese, T. (2002). Elastography : Imaging the elastic properties of soft tissues with ultrasound. *Journal Medicine Ultrasonics*, *29*(Winter), 155–171.

Paluch, Ł., Nawrocka-Laskus, E., Wieczorek, J., Mruk, B., Frel, M., & Walecki, J. (2016). Use of Ultrasound Elastography in the Assessment of the Musculoskeletal System. *Polish Journal of Radiology*, *81*, 240–246.

Park, G., & Kwon, D. (2012). Sonoelastographic evaluation of medial gastrocnemius muscles intrinsic stiffness after rehabilitation therapy with botulinum toxin A injection in spastic cerebral palsy. *Archives of Physical Medicine and Rehabilitation*.

Park, G., & Kwon, D. (2012). Sonoelastographic evaluation of medial gastrocnemius muscles intrinsic stiffness after rehabilitation therapy with botulinum toxin a injection in spastic cerebral palsy. *Archives of Physical Medicine and Rehabilitation*, *93*(11), 2085–2089.

Sikdar, S., Shah, J., Gebreab, T., Yen, R., Gilliams, E., Danoff, J., & Gerber, L. (2009). Novel Applications of Ultrasound Technology to Visualize and Characterize Myofascial Trigger Points and Surrounding Soft Tissue. *Archives of Physical Medicine and Rehabilitation*, *90*(11), 1829–1838.

Sjogaard, G., & Saltin, B. (1982). Extra- and intracellular water spaces in muscles of man at rest and with dynamic exercise. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *243*(3), R271 LP-R280.

Taljanovic, M., Melville, D., Klauser, A., Latt, L., Arif-Tiwari, H., Gao, L., & Witte, R. (2015). Advances in Lower Extremity Ultrasound. *Current Radiology Reports*, *3*(6), 19.

Taş, S., Yılmaz, S., Onur, M., Soylu, A., Altuntaş, O., & Korkusuz, F. (2017). Patellar tendon mechanical properties change with gender, body mass index and quadriceps femoris muscle strength. *Acta Orthopaedica et Traumatologica Turcica*, *51*(1), 54–59.

Tieleman, A., Vinke, A., Alfen, N., Dijk, J., Pillen, S., & Engelen, B. (2012). Skeletal muscle involvement in myotonic dystrophy type 2. A comparative muscle ultrasound study. *Neuromuscular Disorders*, *22*(6), 492–499.

Toledo, M. (2016). *Reliability of ultrasound imaging measures of soft tissue stiffness using elastography in the posterior aspect of the leg*. Unitec.

Vasilescu, D., Vasilescu, D., Dudea, S., Botar-Jid, C., Sfrângeu, S., & Cosma, D. (2010). Sonoelastography contribution in cerebral palsy spasticity treatment assessment, preliminary report: a systematic review of the literature apropos of seven patients. *Medical Ultrasonography*, *12*(4), 306–310.

Yanagisawa, O., Kudo, H., Takahashi, N., & Yoshioka, H. (2004). Magnetic resonance imaging evaluation of cooling on blood flow and oedema in skeletal muscles after exercise. *European Journal of Applied Physiology*, *91*(5–6), 737–740.

Yanagisawa, O., Niitsu, M., Kurihara, T., & Fukubayashi, T. (2011). Evaluation of human muscle hardness after dynamic exercise with ultrasound real-time tissue elastography: A feasibility study. *Clinical Radiology*, *66*(9), 815–819.

Yanagisawa, O., Sakuma, J., Kawakami, Y., Suzuki, K., & Fukubayashi, T. (2015). Effect of exercise-induced muscle damage on muscle hardness evaluated by ultrasound real-time tissue elastography. *SpringerPlus*, *4*(1), 308.

Chapter 4: Vastus lateralis stiffness assessed with supersonic shear wave elastography

Supersonic Shear Image (SSI) is an US elastography method that offers a direct quantitative measure of tissue stiffness based on the velocity of shear waves. In recent years, this technique has become very popular for the study of muscle mechanics in vivo and in human participants. The potential of this technique is enormous and has enabled researchers in many fields, including biomechanics and muscle physiology to probe the function of complex multiarticular muscle groups in passive and active conditions.

In this chapter, we describe a preliminary study using SSI to investigate changes in the stiffness of the VL as a consequence of passive muscle elongation, force production, and repeated muscle activity.

4.1 Effect of knee angle, contractile activity and intensity of force production on vastus lateralis' stifness: a supersonic shear wave elastography study

The recent developments in US imaging modalities have enhanced our ability for studying muscle tissue mechanical properties in vivo, including changes in muscle stiffness associated with stretching and contraction (Gennisson et al., 2010; Koo et al., 2014; Miyamoto et al., 2015). One such advances is SSI (Gennisson et al., 2013). This US modality allows real-time quantification of muscle tissue stiffness in dynamic and relatively unconstrained conditions (Pedersen et al., 2012; Ryu & Jeong, 2017; Smajlovic et al., 2011). The feasibility of SSI to investigate the mechanical properties of the muscle tissue is well documented, including in response to passive muscle elongation (Koo et al., 2014), as well as during isometric contractions of different intensities (Ateş et al., 2015). The good sensitivity of SSI measurements of tissue stiffness has also been explored for addressing questions related with muscle adaptations to resistance training and stretching (Akagi et al., 2016; Nakamura et al., 2014; Umegaki et al., 2015), muscle co-ordination (Ishikawa et al., 2015; Raiteri et al., 2016), and muscle tissue changes caused by ageing (Akagi et al., 2015; Eby et al., 2015) or injury and disease (Lacourpaille et al., 2014). Compared to other techniques, such as the use of hardness meters (Murayama et al., 2012) or torque-angle curves determination (Nordez et al., 2006), US elastography, in particular SSI, allows measuring muscle tissue stiffness in several places of muscles and tendons both at rest and during activity.

Supersonic shear wave imaging is based on measuring the velocity of propagating mechanical vibrations or shear waves generated by focused US beams. This technique uses acoustic radiation force to generate a series of pushes inside the tissue and ultrafast US acquisition for detecting and measuring the propagation of the induced shear waves (Bercoff et al., 2004; Gennisson et al., 2013). The generated planar shear wave propagates with a velocity that is directly proportional to the stiffness of the medium. The mathematical relation describing the velocity of the traveling shear waves is then inverted to construct a map of the Young's modulus (Gennisson et al., 2010). The anisotropic character of the muscle tissue influences the propagation velocity of the shear waves and the sensitivity of SSI to measure muscle stiffness. Thus, the velocity of the shear waves during muscle contraction bear a direct relationship with the intensity of the contraction only when the shear waves propagate parallel to the

length of the muscle fascicles, otherwise saying, when the principal axis of the US probe is oriented parallel to the muscle fibres (Gennisson et al., 2010; Le Sant et al., 2015).

Measuring muscle stiffness is important for understanding muscle function and to monitor muscle status. Muscle stiffness can be an indicator of the length of the muscle, as well as of its contraction status. Good estimates of tibialis anterior elasticity can be obtained with SSI (Koo et al., 2014). In the hamstring muscles, stretching also causes a linear increase in muscle shear modulus (Le Sant et al., 2015). In upper extremity muscles, shear wave velocity is linearly related with the degree of isometric submaximal contractions (<60% MVC) (Gennisson et al., 2010; Yoshitake et al., 2014), which may extend to the full range of isometric torque capacity in the case of small hand muscles (Ateş et al., 2015).

Repeated muscle contractions are also susceptible to increase muscle stiffness that can be detected by SSI (Ishikawa et al., 2016; Lacourpaille et al., 2014; Nordez et al., 2009). Recently, it has been reported that 60 min after a series of isokinetic Ecc contractions (3 sets of 10 contractions at 120°.s^{-1}) there was a significant increase in shear elasticity in the elbow flexors that normalised after 48 hours, except when measured at a more extended elbow angle (i.e., 160°) (Lacourpaille et al., 2014). This Ecc exercise-induced acute increase in shear elasticity was dissociated from fluid accumulation as indicated by comparing the measured transverse relaxation time T_2 with the shear modulus (Lacourpaille et al., 2014).

Muscle shear modulus data has been obtained mostly from arm and calf muscles and less from pennate thigh muscles, like the quadriceps femoris (Dubois et al., 2015). The relationship between the measured shear modulus and the mechanical status of the muscle is more accurate when the probe's main axis is parallel to the direction of the underlying muscle fascicles but can be affected by the obliquity between the probe and the muscle fascicles within the plane of the US scan (Miyamoto et al., 2015). Also, there is a lack of information regarding the immediate effect of repeated muscle contractions on muscle stiffness and particularly when such activity combines different modes of contraction. Therefore, we conducted a preliminary SSI study to assess changes in VL's shear modulus with knee position and after a session of maximal isometric and isokinetic Conc and Ecc contractions. The relationship between VL's shear elasticity and submaximal knee extension torque was also investigated.

4.1.1 Materials and Methods

Participants

Sixteen young and active subjects, ten males, (mean ± SD; height: 1.69 ± 0.07 m, weight: 66.7 ± 8.1 kg, age: 20.06 ± 2.02 years) participated in this study. Participants were fully informed of the purpose and the procedures of the study and signed a written informed consent.

Protocol

Data were collected in a single session. All contractions were performed on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems). Participants sat on the dynamometer chair with the trunk stabilized with straps crossing the chest and the pelvis. The right leg was fixed to the dynamometer's arm immediately above the malleoli and the knee joint was aligned with the rotating center of the dynamometer arm. Participants remained seated on the dynamometer during the entire session.

After a warm-up consisting on a few submaximal isometric, Conc, and Ecc contractions, participants performed a series of maximal contractions with the knee extensors that begun with 3 repetitions of isometric MVCs at 30°, 60°, and 90° of knee flexion, followed by 2 sets of maximal Conc and Ecc contractions at angular velocities of 120, 90, and 60°.s^{-1} and performed through a ROM between 90° and 0° of knee flexion (0° - total knee extension). To match the time muscles were active during the different contraction velocities, the number of repetitions was 6, 4, and 2 for sets performed at angular velocity of 120°.s^{-1}, 90°.s^{-1}, and 60°.s^{-1}, respectively. The order of the maximal isometric, Conc and Ecc contractions was randomized and counterbalanced between the participants. During dynamic contractions, the order of the sets run from the highest to the lowest angular velocity (from 120°.s^{-1} down to 60°.s^{-1}), for Conc contractions, and in the opposite sense (from 60°.s^{-1} up to 60°.s^{-1}) for Ecc contractions. A computer monitor facing the participants provided feedback about the torque produced during the contractions. Verbal encouragement was also given during the efforts. A 2 min rest separated each isometric MVC and each contraction set.

After 5 min of resting, two isometric ramp contractions were performed between 0 and 60% of isometric MVC and at 60° of knee flexion. Participants received visual feedback about the time and the torque generated and were requested to increase the latter from 0 to 60% MVC in about 10 s.

Shear wave elastography

An Aixplorer US equipment (version 4.2; Supersonic Imagine, Aix-en-Provence, France) equipped with a 4-15 MHz linear transducer array (SuperLinear 15-4, Vermon, Tours, France) in shear wave elastography mode and musculoskeletal preset was used for SSI muscle scanning (Figure 25).

The US probe was placed over the VL at a position corresponding to 39% of the distance between the upper edge of the patella and the anterior superior iliac spine (Blazevich et al., 2006). B-mode images were used to align the principal axis of the probe with the direction of the muscle fascicles. The probe orientation was considered optimal when the hyperechoic images corresponding to the muscle fascicles were clearly visible and few fascicles within the belly of the VL could be seen in their full length. Ultrasound gel was applied to ensure acoustic coupling. After anatomical location and probe orientation have been set, skin landmarks were drawn on the skin to guarantee that the probe was placed over the same region of the VL during repeated scans. Measurements of shear wave modulus were made with the knee at 10, 50, and 90° flexion. Participants were asked to remain as relaxed as possible during all static measurements.

The shear wave elastography field of view was delimited by a fixed-size square ROI (1.5 cm^2) placed within the VL and away from fibrous and adipose septa.

Data Processing

For each measurement, the acquisition was made as a video of shear wave elastography maps (mp4 format) that were next transformed into a sequence of 'jpeg' images.

Using a Matlab routine (R2013a, The MathWorks Inc., Natick, USA), the images were decomposed into the three red-green-blue matrices. The shear modulus (μ) is calculated assuming a linear elastic behavior of the tissues and using the following formula: $\mu = pVs^2$ (where p is the muscle mass density 1000 kg/m^3). The intensity of the pixels was quantified and converted to kPa values, considering that the highest pixel values were red and the lowest blue. The saturating shear modulus was above 100 kPa.

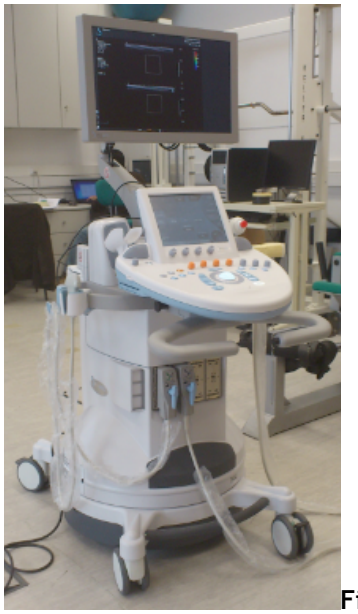


Figure 25: Schematic representation of the ultrasound equipment with supersonic shear wave elastography capability.

Statistical Analysis

Two-way within-subjects' ANOVA was used to compare the shear modulus before and after knee extension contractions and between the knee joint angles. The same analysis was also used to evaluate the effect of the type of contraction (i.e., isometric, Conc, and Ecc). The sphericity assumption was tested with Mauchly's test and in cases this assumption was violated, the Greenhouse-Geiser correction for the significance level was considered. Polynomial contrasts were calculated using one-way ANOVA for shear modulus values during ramp contractions. The determination coefficient (R^2) was derived using linear regression analysis. All statistical tests were conducted using SPSS software package, v. 22 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD. Statistical significance was accepted at $p<0.05$.

4.1.2 Results

Shear modulus values during ramp contractions could not be measured from one subject.

At baseline, the values for the relaxed VL's shear modulus were 5.06 ± 1.48, 5.68 ± 2.08, and 9.40 ± 4.10 kPa at 10, 50 and 90° knee flexion, respectively. By the end of the study, these values had risen to 5.87 ± 2.83, 7.05 ± 3.65 and 10.95 ± 2.92 kPa. ANOVA revealed a significant pre-post [$F_{(1,14)}=8.122$; $p=0.013$] and knee angle [$F_{(2,28)}=43.467$; $p<0.001$] effects on shear wave elasticity but without any interaction effect between these two factors [$F_{(1,14)}=0.160$; $p=0.853$]. Compared to baseline, the shear modulus increased after isometric [$F_{(2,28)}=9.354$; $p=0.009$] and Ecc [$F_{(2,28)}=6.512$; $p=0.023$] contractions, but not after Conc contractions [$F_{(2,28)}=4.358$; $p=0.056$]. In all cases, the

interaction effect between the factors pre-post and knee angle was not significant. No differences were found between the shear modulus measured at the end of each contraction type (Table 18).

Table 18: Two-way within-subjects ANOVA results for shear modulus data.

Two-way within-subjects ANOVA results for shear modulus data									
Test	Factor pre vs. post			Factor knee angle			Interaction effect		
	Degrees of freedom Factor; Error	F-ratio	*p* value	Degrees of freedom Factor, Error	F-ratio	*p* value	Degrees of freedom Factor; Error	F-ratio	*p* value
Pre vs. post all contractions	2; 28	43.467	0.000	1; 14	8.122	0.013	2; 28	0.160	0.853
Pre vs. post isometric	2; 28	31.067	0.000	1; 14	9.356	0.009	2; 28	1.67	0.847
Pre vs. post concentric	2; 28	43.675	0.000	1; 14	4.358	0.056	2; 28	1.516	0.237
Pre vs. post eccentric	2; 28	25.612	0.000	1; 14	6.512	0.023	2; 28	0.963	0.394

During ramp contractions, shear wave velocity increased linearly with torque production (Figure 26). The mean of the individual R^2 (coefficient of determination) values, calculated from the regression analysis for the values of muscle torque and shear modulus and without considering the resting values, reached 0.77 ± 0.33 (range: 0.27-0.99) (Figure 27). The results of the polynomial contrasts show a significant linear effect between the VL's shear modulus and the percentage of maximal knee extension torque production [$F_{(1,14)}$=37.934; p<0.001]. When testing for a quadratic relationship between these two variables, the same analysis showed that the effect was not significant [$F_{(1,14)}$=0.512; p<0.482], which can be interpreted as showing that, at least at up to 60% isometric maximal knee extension torque, there was no levelling off in the increase of the VL's shear modulus.

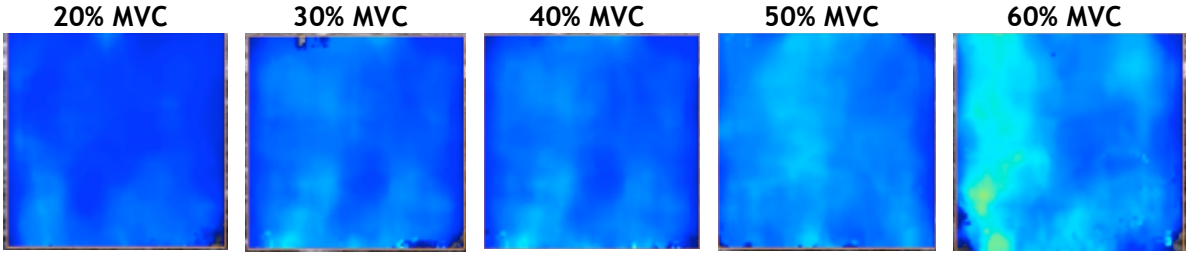
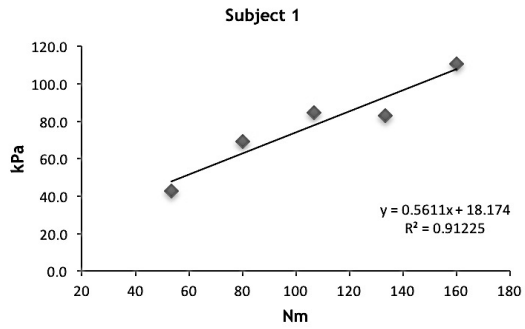
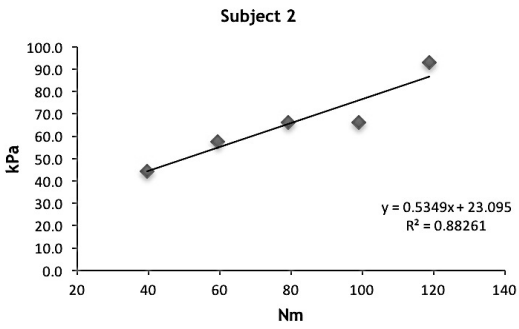
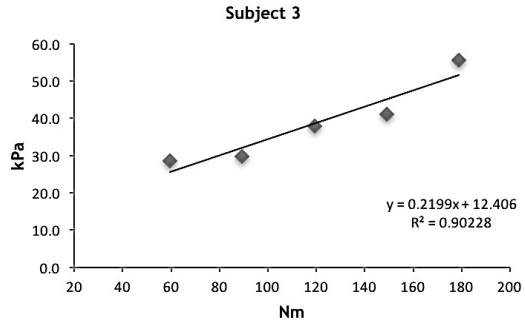
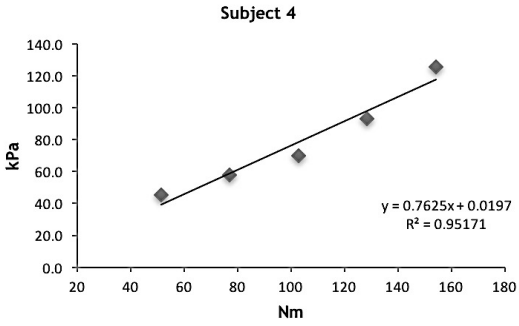


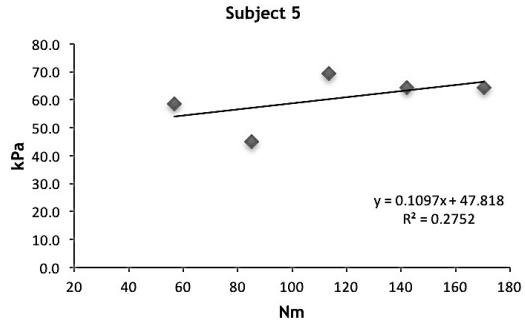
Figure 26: Typical shear wave elastograms during ramp isometric contractions of increasing intensity.

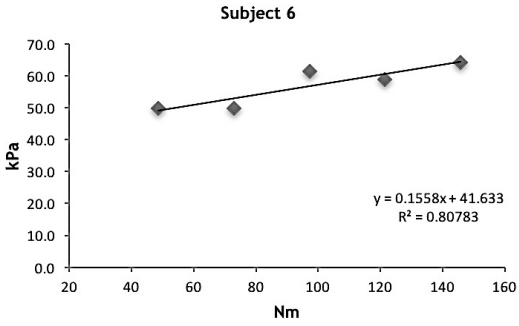

Subject 1
kPa
Nm
y = 0.5611x + 18.174
R² = 0.91225

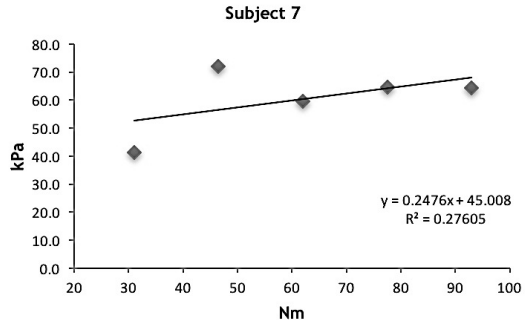

Subject 2
kPa
Nm
y = 0.5349x + 23.095
R² = 0.88261

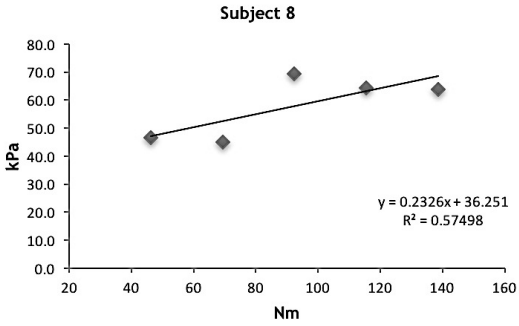

Subject 3
kPa
Nm
y = 0.2199x + 12.406
R² = 0.90228

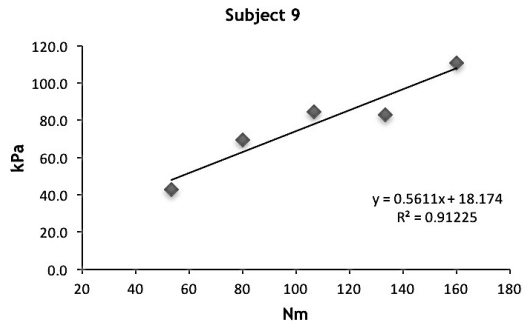

Subject 4
kPa
Nm
y = 0.7625x + 0.0197
R² = 0.95171

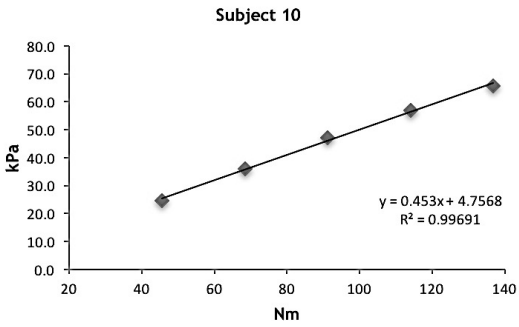

Subject 5
kPa
Nm
y = 0.1097x + 47.818
R² = 0.2752


Subject 6
kPa
Nm
y = 0.1558x + 41.633
R² = 0.80783


Subject 7
kPa
Nm
y = 0.2476x + 45.008
R² = 0.27605


Subject 8
kPa
Nm
y = 0.2326x + 36.251
R² = 0.57498


Subject 9
kPa
Nm
y = 0.5611x + 18.174
R² = 0.91225


Subject 10
kPa
Nm
y = 0.453x + 4.7568
R² = 0.99691

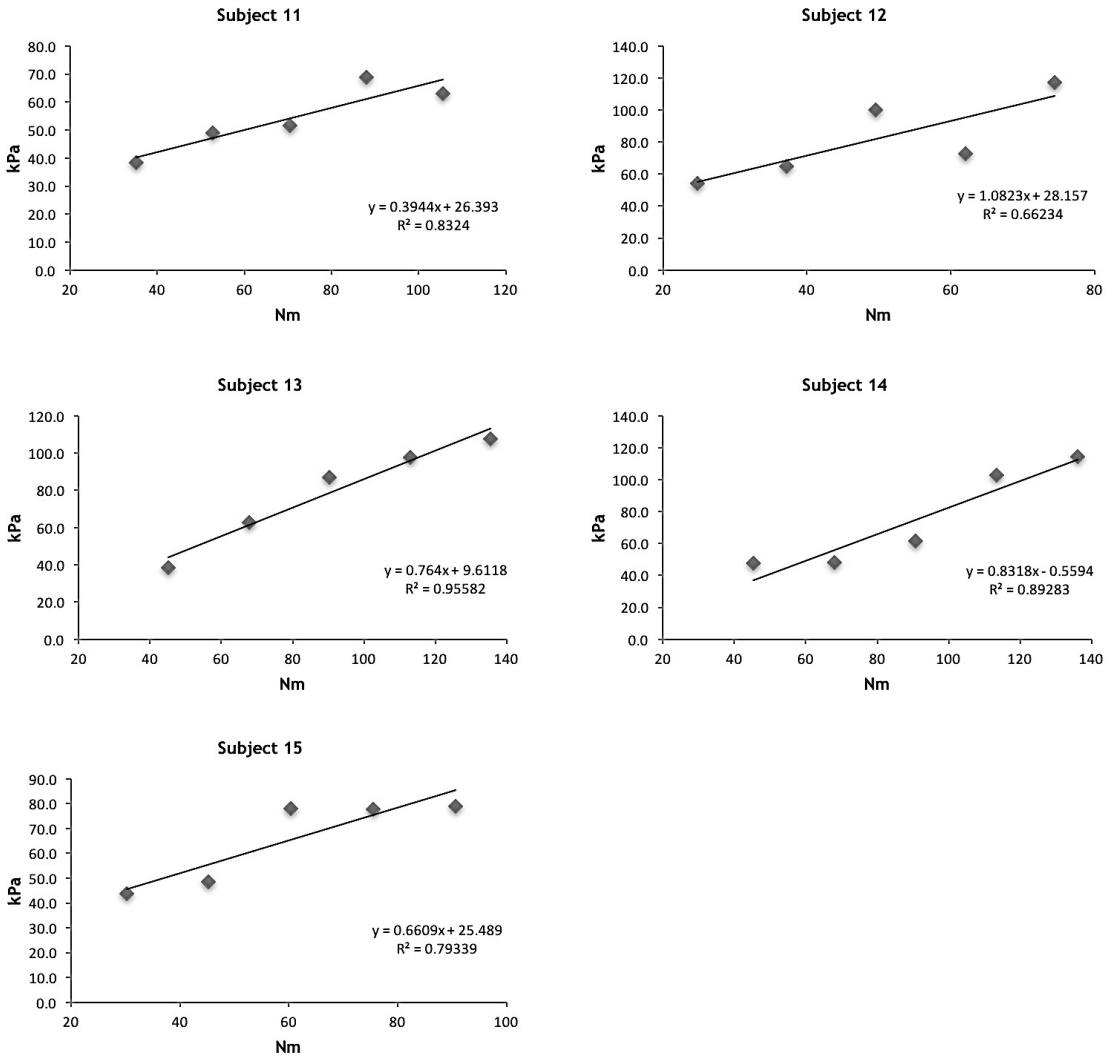


Figure 27: Individual linear regressions between isometric extension torque and shear modulus.

4.1.3 Discussion

In this study, a number of observations regarding changes in VL's shear modulus with muscle length and contraction were obtained, namely that: 1) in the relaxed VL, the shear modulus increases with knee flexion, 2) the VL's shear modulus increases linearly with the degree of isometric contraction, and 3) after a session comprised by isometric, Conc and Ecc maximal contractions, the VL's shear modulus increases. In general, these observations agree with data in the literature.

The presence of an accurate relationship between the length of relaxed muscles and the shear modulus values is reported by several studies and for a number of lower limb muscles (Hug et al, 2013; Koo et al., 2014; Le Sant et al., 2015; Levinson et al, 1995; Nordez et al., 2008). Employing transient US elastography, Nordez et al. (2008) successfully fitted a linear relationship between the measured stiffness of the medial gastrocnemius with the ankle joint angle and with passive ankle torque. In this study, a

mean of 2.6 times increase in medial gastrocnemius stiffness was seen when the ankle was passively moved along an 80º arc (40º plantar flexion to 40º dorsiflexion) (Nordez et al., 2008). A good relationship between the muscles' shear modulus and the degree of knee and hip flexion/extension has also been reported for the hamstrings, although the shear moduli for the passively stretched hamstring muscles were considerably higher than the ones found by us for the VL, ranging between 9.7 and 13.2 kPa when the knee was placed at 30% of the knee ROM and the hip at 90º flexion (Le Sant et al., 2015). The exact relationship between relaxed muscle stiffness and joint excursion may depend on specific morphological parameters of the muscle and the joints (Koo et al., 2014; Maïsetti et al., 2012). In the case of the VL, we found that the increase in the shear modulus with knee flexion was not linear, being larger when the knee was bent from 50º to 90º than between 10º and 50º. This agrees with data from other pennate muscles and may be partially caused by diminished obliquity of the muscles fascicles relative to probe orientation (Miyamoto et al., 2015).

The main purpose of this study was to evaluate the acute effect of intense muscle activity on relaxed muscle stiffness using SSI. Our results clearly demonstrated an acute increase in muscle stiffness caused by previous muscle intense contractile activity, which is in line with previous studies (Akag et al., 2015; Lacourpaille et al., 2014) but not with others (Nordez et al., 2009). In a recent study, three sets of ten maximal isokinetic Ecc contractions increased passive stiffness of the biceps brachii one hour after the end of the contractions (Lacourpaille et al., 2014). Similar findings are reported by Akagi et al. (2015), who have shown increased passive stiffness of the triceps brachii immediately after five sets of eight repetitions of a dumbbell extension exercise performed at 80% of one repetition maximum (Akagi et al., 2015). In both of these two studies the increase in passive stiffness occurring acutely after resistance exercise was unrelated with the severity of the accompanying muscle swelling. These results contrast with the effect of fatiguing submaximal muscle activity on passive muscle stiffness (Andonian et al., 2016; Nordez et al., 2009). After a fatiguing isometric contraction of the plantar flexors, performed at 40% MVC, muscle stiffness of the medial gastrocnemius decreased immediately after the termination of the submaximal exertion (Nordez et al., 2009). In a recent and well-controlled study, passive stiffness of the superficial heads of the quadriceps femoris (i.e., the VL, VM, and RF) was shown to significantly decrease after completing a mountain ultra-marathon (Andonian et al., 2016). Therefore, the impact of muscle activity on muscle stiffness looks as to depend on the relative intensity, the duration, and possible the type of the contractions. In our study, the acute increase in the relaxed VL stiffness was more evident after isometric

and Ecc maximal contractions than after maximal Conc contractions. However, this observation should be interpreted with caution since all types of contractions were performed within the same session.

Another important application of SSI is determining the level of contraction of a single muscle by establishing the relationship between the amount of force (or torque) it produces and its stiffness (Ateş et al., 2015; Gennisson et al., 2005; Yoshitake et al., 2014). This use of SSI is particularly important for studying force sharing between synergistic muscles in many different contexts (Bouillard et al., 2014; Bouillard et al., 2011). In our study, we could also found a rather precise relationship between the amount of isometric knee extension torque and the VL's stiffness. For hand muscles, the precision of the relationship between muscle stiffness and generated torque surpasses that between the surface EMG and torque (Bouillard et al., 2011). In our study, the mean coefficient of determination between VL's stiffness and knee extension torque was 0.77, which is lower than 0.98 the value reported for hand muscles (Bouillard et al., 2011). Indeed, we found a considerable variation in the values of the coefficient of determination amongst our participants. The reasons behind such large variation cannot be totally disclosed but may be related with a higher difficulty in constraining the action of the knee extensors compared to the first dorsal interosseous or the abductor digiti minimi (Bouillard et al., 2011). Also, the ramp contractions in our study were performed at the end of the session and after the maximal contractions, so muscle fatigue could have affected the results (Bouillard et al., 2014).

4.1.4 Limitations

Due to its preliminary nature, this study has a few limitations. The major limitation being that the study was conducted in a single session that included maximal isometric, Conc, and Ecc isokinetic contractions plus ramp contractions. This prevents us from getting strong conclusions from our findings. Also, we just collected data immediately after the session and did not monitor recovery. Another limitation relies on the fact that the probe was manually held by the operator.

4.1.5 Summary

By employing SSI, this study demonstrated that passive stiffness of the VL increases non-linearly with knee flexion as well as after maximal isometric and Ecc contractions, with the acute effect of Conc contractions on VL's passive stiffness being less clear. A rather accurate relationship between the amount of isometric torque and VL's stiffness was

also demonstrated. This study adds to several other studies in revealing the high potential of SSI for studying muscle function in humans.

4.1.6 References

Akagi, R., Shikiba, T., Tanaka, J., & Takahashi, H. (2016). A Six-Week Resistance Training Program Does Not Change Shear Modulus of the Triceps Brachii. *Journal of Applied Biomechanics*, *32*(4), 373–378.

Akagi, R., Tanaka, J., Shikiba, T., & Takahashi, H. (2015). Muscle hardness of the triceps brachii before and after a resistance exercise session: a shear wave ultrasound elastography study. *Acta Radiologica (Stockholm, Sweden : 1987)*, *56*(12), 1487–1493.

Akagi, R., Yamashita, Y., & Ueyasu, Y. (2015). Age-related differences in muscle shear moduli in the lower extremity. *Ultrasound in Medicine and Biology*, *41*(11), 2906–2912.

Andonian, P., Viallon, M., Le Goff, C., Bourguignon, C., Tourel, C., Morel, J., … Croisille, P. (2016). Shear-wave elastography assessments of quadriceps stiffness changes prior to, during and after prolonged exercise: A longitudinal study during an extreme mountain ultra-marathon. *PLoS ONE*, *11*(8).

Ateş, F., Hug, F., Bouillard, K., Jubeau, M., Frappart, T., Couade, M., … Nordez, A. (2015). Muscle shear elastic modulus is linearly related to muscle torque over the entire range of isometric contraction intensity. *Journal of Electromyography and Kinesiology*, *25*(4), 703–708.

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*. https://doi.org/10.1109/TUFFC.2004.1295425

Blazevich, A., Gill, N., & Zhou, S. (2006). Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *Journal of Anatomy*, *209*(3), 289–310.

Bouillard, K., Jubeau, M., Nordez, A., & Hug, F. (2014). Effect of vastus lateralis fatigue on load sharing between quadriceps femoris muscles during isometric knee extensions. *Journal of Neurophysiology*, *111*(4), 768–776.

Bouillard, K., Nordez, A., & Hug, F. (2011). Estimation of individual muscle force using elastography. *PLoS ONE*, *6*(12).

Dubois, G., Kheireddine, W., Vergari, C., Bonneau, D., Thoreux, P., Rouch, P., … Skalli, W. (2015). Reliable Protocol for Shear Wave Elastography of Lower Limb Muscles at Rest and During Passive Stretching. *Ultrasound in Medicine and Biology*, *41*(9), 2284–2291.

Eby, S., Cloud, B., Brandenburg, J., Giambini, H., Song, P., Chen, S., … An, K. (2015). Shear wave elastography of passive skeletal muscle stiffness: Influences of sex and age throughout adulthood. *Clinical Biomechanics*, *30*(1), 22–27.

Gennisson, J., Deffieux, T., Fink, M., & Tanter, M. (2013). Ultrasound elastography: principles and techniques. *Diagnostic and Interventional Imaging*, *94*(5), 487–95.

Gennisson, J., Cornu, C., Catheline, S., Fink, M., & Portero, P. (2005). Human muscle hardness assessment during incremental isometric contraction using transient elastography. *Journal of Biomechanics*, *38*(7), 1543–1550.

Gennisson, J., Deffieux, T., Macé, 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 and Biology*, *36*(5), 789–801.

Hug, F., Lacourpaille, L., Maïsetti, O., & Nordez, A. (2013). Slack length of gastrocnemius medialis and Achilles tendon occurs at different ankle angles. *Journal of Biomechanics*, *46*(14), 2534–8.

Ishikawa, H., Muraki, T., Morise, S., Sekiguchi, Y., Yamamoto, N., Itoi, E., & Izumi, S. (2016). Changes in stiffness of the dorsal scapular muscles before and after computer work: a comparison between individuals with and without neck and shoulder complaints. *European Journal of Applied Physiology*.

Ishikawa, H., Muraki, T., Sekiguchi, Y., Ishijima, T., Morise, S., Yamamoto, N., … Izumi, S. (2015). Noninvasive assessment of the activity of the shoulder girdle muscles using

ultrasound real-time tissue elastography. *Journal of Electromyography and Kinesiology*, *25*(5), 723–730.

Bouillard, K., Nordez, A., & Hug, F. (2011). Estimation of Individual Muscle Force Using Elastography. *PLoS ONE*, *6*(12), e29261.

Koo, T., Guo, J., Cohen, J., & Parker, K. (2014). Quantifying the passive stretching response of human tibialis anterior muscle using shear wave elastography. *Clinical Biomechanics (Bristol, Avon)*, *29*(1), 33–9.

Lacourpaille, L., Hug, F., Guevel, A., Pereon, Y., Magot, A., Hogrel, J., & Nordez, A. (2014). New insights on contraction efficiency in patients with Duchenne muscular dystrophy. *Journal of Applied Physiology*, *117*(6), 658–662.

Lacourpaille, L., Nordez, A., Hug, F., Couturier, A., Dibie, C., & Guilhem, G. (2014). Time-course effect of exercise-induced muscle damage on localized muscle mechanical properties assessed using elastography. *Acta Physiologica*, *211*(1), 135–146.

Le Sant, G., Ates, F., Brasseur, J., & Nordez, A. (2015). Elastography study of hamstring behaviors during passive stretching. *PLoS ONE*, *10*(9), 1–13.

Levinson, S., Shinagawa, M., & Sato, T. (1995). Sonoelastic determination of human skeletal-muscle elasticity. *Journal of Biomechanics*, *28*(10), 1145–54.

Maïsetti, 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*(6), 978–984.

Miyamoto, N., Hirata, K., Kanehisa, H., & Yoshitake, Y. (2015). Validity of measurement of shear modulus by ultrasound shear wave elastography in human pennate muscle. *PLoS ONE*, *10*(4), 1–11.

Murayama, M., Watanabe, K., Kato, R., Uchiyama, T., & Yoneda, T. (2012). Association of muscle hardness with muscle tension dynamics: A physiological property. *European Journal of Applied Physiology*, *112*(1), 105–112.

Nakamura, M., Ikezoe, T., Kobayashi, T., Umegaki, H., Takeno, Y., Nishishita, S., & Ichihashi, N. (2014). Acute effects of static stretching on muscle hardness of the medial gastrocnemius muscle belly in humans: An ultrasonic shear-wave elastography study. *Ultrasound in Medicine and Biology*, *40*(9), 1991–1997.

Nordez, A., Cornu, C., & McNair, P. (2006). Acute effects of static stretching on passive stiffness of the hamstring muscles calculated using different mathematical models. *Clinical Biomechanics*, *21*(7), 755–760.

Nordez, A., Gennisson, J., Casari, P., Catheline, S., & Cornu, C. (2008). Characterization of muscle belly elastic properties during passive stretching using transient elastography. *Journal of Biomechanics*, *41*(10), 2305–2311.

Nordez, A., Guével, A., Casari, P., Catheline, S., & Cornu, C. (2009). Assessment of muscle hardness changes induced by a submaximal fatiguing isometric contraction. *Journal of Electromyography and Kinesiology*, *19*(3), 484–491.

Pedersen, M., Fredberg, U., & Langberg, H. (2012). Sonoelastography as a diagnostic tool in the assessment of musculoskeletal alterations: a systematic review. *Ultraschall Medicine*, *33*(5), 441–6.

Raiteri, B., Hug, F., Cresswell, A., & Lichtwark, G. (2016). Quantification of muscle co-contraction using supersonic shear wave imaging. *Journal of Biomechanics*, *49*(3), 493–495.

Ryu, J., & Jeong, W. (2017). Current status of musculoskeletal application of shear wave elastography. *Ultrasonography (Seoul, Korea)*, 1–13.

Smajlovic, F., Carovac, A., & Bulja, D. (2011). Sonoelastography : the method of choice for evaluation of tissue elasticity. *Journal of Health Sciences*, *1*(1), 50–55.

Umegaki, H., Ikezoe, T., Nakamura, M., Nishishita, S., Kobayashi, T., Fujita, K., … Ichihashi, N. (2015). Acute effects of static stretching on the hamstrings using shear elastic modulus

determined by ultrasound shear wave elastography: Differences in flexibility between hamstring muscle components. *Manual Therapy*, *20*(4), 610–613.

Yoshitake, Y., Takai, Y., Kanehisa, H., & Shinohara, M. (2014). Muscle shear modulus measured with ultrasound shear-wave elastography across a wide range of contraction intensity. *Muscle and Nerve*, *50*(1), 103–113.

Yoshitake, Y., Takai, Y., Kanehisa, H., & Shinohara, M. (2014). Muscle shear modulus measured with ultrasound shear-wave elastography across a wide range of contraction intensity. *Muscle & Nerve*, *50*(1), 103–13.

Chapter 5: General discussion

This general discussion is organised in three main points: 1) discussion of main findings, 2) methodological issues and limitations, and 3) implications for future research.

5.1 Discussion of the main findings

In this thesis, we aimed to investigate the reproducibility of ultrasound imaging for studying MT, EI and muscle stiffness and provided data regarding the response of these parameters to strength training. In addition, we conducted a preliminary study using SSI for investigating acute changes in VL' stiffness in response to the level of muscle contraction, passive stretching, and high-intensity muscle activity.

5.1.1 The reproducibility of MT and EI measurements of the quadriceps femoris

Previous studies show that both MT and EI measures are highly reliable. The results of our study, reported in Chapter 2, also reveal a high level of reproducibility for MT and EI measured from the different heads of the quadriceps femoris. We evaluated reproducibility by calculating the ICC. Although the ICC is often used as a measure of reliability, its value depends on the population in which measurements are made and not just on the measurement error of the method itself. The ICC is a measure of the correlation between any two measurements made on the same subject (values: 0-1) and therefore is not a measure of accuracy. To improve the assessment of reproducibility, we also calculated the SEM and SDC, which give us more direct information regarding the precision of the measure, since these parameters are in the same units as the measure itself. In addition, we have also conducted agreement analysis. The agreement analysis quantifies how close two measurements in the same scale and collected from the same subject are (Bartlett & Frost, 2008). Overall, the level of reproducibility gives the expected minimum amount of variation in measurements collected from a single subject or from a sample under changing conditions (different measurement methods or instruments, measurements by different observers, or over a period of time) that can be considered 'error-free' and an indication of a non-negligible change. In general, the ICC and agreement values are inversely proportional (Bartlett & Frost, 2008).

In this study, a high to very high intra- and inter-session reliability for MT and EI measures could be demonstrated. This is in line with the literature (Agyapong-Badu et al., 2014; Blazevich et al., 2006; Caresio et al., 2014; Boer et al., 2008; Fukumoto et al., 2012). For MT, the results showed high to very high intra- and inter-session ICCs.

Comparing to transverse scans, MT measured in the longitudinal scans are somewhat less reliable, displaying slightly larger SEM and SDC values, several other studies report similar results (Mangine, 2014; Palmer, 2015; Raj et al., 2012; Strasser et al., 2013). In this study, EM values for MT measurements were similar across the whole quadriceps femoris, however, the SDC values for MT measures showed considerable variation across this muscle, which is corroborate by the literature (Jhu et al., 2010; Koppenhaver et al., 2009).

The reliability of our ultrasound MT measures appeared to be slightly better in transverse scans than in longitudinal ones. Transverse scans offer a better visualization of the anatomical details of the several quadriceps femoris heads and based in our data should be used to measure MT. Some authors defend that longitudinal scans are better for studying the skeletal muscle (Blazevich et al., 2006; Konig et al., 2014; Wilhelm et al., 2014), but others use transverse views (Agyapong-Badu et al., 2014; Boer et al., 2008; Fukumoto et al., 2012). Our results also show that MT is underestimated by 0.2 cm when measured from longitudinal scans, compared with measurements made on transverse scans. Only a few studies conducted Bland–Altman analysis (including mean differences) for ultrasound MT measurements (English et al., 2012).

The results for EI measures were also found to be highly reliable, both when collected in the same session or in sessions one-week apart. However, for inter-session reliability, ICCs were higher using the whole muscle image as a ROI. Similar findings were obtained by some authors (Caresio et al., 2015; Fukumoto et al., 2012; Radaelli et al., 2012).

The study reported in Chapter 2 was conducted in young adults not engaged in sports or intense physical activities and the results showed higher MT values for VM and RF and lower MT values for VL and VI. Comparing with the literature, our data is similar with the values obtained by Strasser et al. (2013) but lower than the values reported by Ruas et al. (2017) (Ruas et al., 2017; Strasser et al., 2013). The lack of physical activity or sedentary lifestyle can influence these results, which together with an unhealthy diet can be associated with low MT values and high echo-intensities, revealing poor muscle conditioning. The differences found between authors may be due to the use of different equipment settings, which is still a strong limitation in the evaluation of muscle EI. However, in our study as well as in other studies, it is concluded that the VM muscle is the head of the quadriceps femoris with the highest MT, whereas the VI is the one presenting the lowest one. As for the EI, the VL muscle showed the highest value, presenting more connective and fat tissue and suggesting less recruitment of this muscle during daily activities (Ruas et al., 2017; Strasser et al., 2013).

5.1.2 The effect of strength training on quadriceps femoris' MT, EI, and stiffness

One important application of ultrasound imaging is assessing muscle adaptation to different kinds of stimuli, in particular to strength training (Branderburg & Docherty, 2006; Herrick et al., 2017). This includes the ability to evaluate changes in muscle size, through MT measurements, and of muscle quality, through measures of EI (Jansen et al., 2012).

Quantitative assessment of EI is a relatively easy method for studying muscle quality and its changes have been related with ageing, neuromuscular disorders, and muscle conditioning (Cadore et al., 2014; Fukumoto et al., 2012; Jenkins et al., 2015). In the past, EI has been graded visually, but this approach is subjective and depends on the experience of the observer, and therefore is now considered unacceptable (Pillen et al., 2006). To overcome this limitation, computer-aided grayscale analysis has been implemented for quantitative measurement of skeletal muscle EI (Fukumoto et al., 2012; Pillen et al., 2006; Pillen et al., 2009). Quantitative evaluation of US images is preferable over visual evaluation, because it is more sensitive and objective and offers the possibility to perform statistical analysis. A prerequisite for the diagnostic use of quantitative muscle US is the availability of normal reference values. Reference values for EI are available for various muscles (Cruz-Montecinos et al., 2016; Ruas et al., 2017; Verhulst et al., 2011). Echo-intensity is quantified by averaging the grayscale value of each individual pixel in a defined ROI from an ultrasound image (Varanoske et al., 2017). Consequently, the quantitative estimation of EI in clinical settings is often through grayscale histogram analysis. This imaging analysis technique involves the construction of a plot featuring the number of pixels associated with a given ROI within intervals determined by intensity level. Post-image acquisition analysis may be performed using a variety of image editing programs (Harris-Love et al., 2016; Pillen et al., 2009).

In Chapter 3, participants were submitted to a strength training program and performed either Conc or Ecc training over 15 weeks. The results showed significant changes in MT and EI measures in response to training, but with notable differences between the different heads of the quadriceps femoris. However, no differences in MT and EI responses were found as a result of the two types of training. Particularly surprising was the fact that no changes in MT were seen after strength training in the lower and middle regions of the VM. This observation cannot be justified by poor reliability, since we have shown in Chapter 2 that the MT measures collected from the VM possess ICC, SEM and SDC values that in general are better than in the remaining regions of the

quadriceps femoris (see Chapter 2, Table 5). The characteristics of the strength training program might explain the lack of MT increase in the VM. The fact that loading progression was largely based on increasing contractions' velocity and not the amount of produced torque might have affected the hypertrophic response, at least in some of the knee extensor muscles. Similar findings were also seen for EI measures. In fact, no changes in EI as a result of strength training could be found in the VM, but in this case EI was also unchanged in the RF and in the upper region of the VL. The VI showed the most consistent changes in EI in response to strength training, with a decrease in EI values over the 15 weeks of training. Once again, poor reliability does not explain these findings, since the VI is the head of the quadriceps femoris with highest SDC values for EI measurements (see Chapter 2, Tables 6 and 7).

Muscle EI is believed to mirror the relative amount of fat and connective tissue composing the scanned structure and, for instance with aging, increases in EI are interpreted as mirroring the loss of the muscle tissue. Therefore, we could expect a decrease in muscle EI following strength training as a result of muscle hypertrophy. However, such increase could not be found, except for the VI, even when a significant increase in MT existed. The likely explanation is that muscle hypertrophy is accompanied by a matched increase in the amount of connective tissue so that the relative amount of connective and muscle tissue does not change with strength training.

Muscle depth is also a factor that may influence muscle's EI values. During data collection, a single frequency was used that allowed to visualize the entire quadriceps femoris. However, with increasing depth the ultrasound beam loses its capacity of penetration and image resolution diminishes. Therefore, deep seated muscles, such as the VI, may show lower EI simply because they are located in deeper regions of the limb.

In Chapter 3, study 2, we report data of VL's stiffness obtained by QSE and the results show increased stiffness as a result of strength training, although without differences between Conc and Ecc training. Also, we could corroborate observations made by Drakonaki et al. (2012) that characterise the VL as an heterogeneous tissue in terms of stiffness, with patchy areas of red colour surrounded by areas of predominant green and blue colours in line with (Drakonaki et al., 2012). Several studies already showed the viability of the QSE to assess the muscle stiffness (Brandenburg et al., 2014; Hirono et al., 2016; Niitsu et al., 2011) but this study is the first to demonstrate an increase in muscle stiffness as a result of strength training by means of ultrasound elastography.

However, our observations should be interpreted with caution. In this study, we have found only moderate intra-evaluation reliability for colour mapping values by QSE ultrasound. In addition, SEM and SDC values were relatively large. Furthermore, this technique only offers semi-quantitative measures of muscle stiffness and the values should be compared to a reference structure and reported as a ratio measure, which was not done in this thesis. While QSE has many limitations, it is still employed to assess muscle function in several contexts also because it is one of the first ultrasound elastography methods implemented in commercial machines (Brandenburg et al., 2014; Chino et al., 2012; Hirono et al., 2016; Muraki et al., 2014; Niitsu et al., 2011; Yanagisawa et al., 2011).

5.1.3 The use of ultrasound elastography for measuring vastus lateralis' stiffness

In this thesis, two different types of elastography were used to assess stiffness of the VL. In Chapter 4 changes in VL's stiffness resulting from passive stretching, isometric contraction, and strong contractile activity were investigated using SSI elastography. This method measures shear wave velocity and derives the shear wave modulus, which gives a quantitative measure of tissue stiffness. Our results clearly showed an acute increase in stiffness of the relaxed VL after a few sets of maximal isometric, Conc, and Ecc contractions and in response to muscle stretching caused by changing knee flexion angle. Finally, a reasonably good linear relationship was found between the level of isometric contraction and VL's stiffness. These results are in line with those reported in other studies and confirm the great potential of SSI for studying muscle function in a variety of conditions (Hug et al, 2013; Koo et al., 2014; Le Sant et al., 2015; Levinson et al, 1995; Nordez et al., 2008).

Unfortunately, the preliminary character of the SSI study prevented the study design to comply with a number of methodological requisites for a well-controlled study.

5.2 Methodological issues and limitations

Many of the limitations in this thesis were discussed in the respective chapters and will not be mentioned again here. However, there are a few issues and study limitations that must be considered at this point.

5.2.1 Participants' characteristics

In all our studies, only young, healthy participants were recruited. While this is advantageous because it eases recruitment, it limits the generalizability of the findings to other age groups.

5.2.2 Confounding variables

Efforts were made to minimise any biasing effects caused by potential confounding variables and these included the use of a control group in the strength training study and implementing a counterbalanced design in the SSI study. Increased reliability was also achieved by having an experienced operator performing every ultrasound data collection. However, during ultrasound scans the probe was held manually. Although manual operation is often required for fine adjustments of probe orientation and for more precise probe placement, it can also be a source of enhanced variation or may even introduce bias caused, for instance, by operator's fatigue.

5.2.3 Equipment and validation

Three ultrasound machines were used to collect the data, which constrains the ability to compare our results. Nevertheless, MT and EI data reported in Chapter 2 and 3 were comparable, both in qualitative and quantitative terms, although they were collected with two different ultrasound machines. A further limitation regards the validation of our data using a "gold standard" reference. For ultrasound imaging, magnetic resonance imaging could have been use to validate MT measures, whereas hardmeter devices or passive muscle-torque curves could have been used to validate the ultrasound elastography-derived measures of muscle stiffness.

5.2.4 Implications for future research

Regarding reproducibility of quantitative ultrasound, future research should focus on quality control of the ultrasound acquisition in order to improve reproducibility across different scanners. The interpretation of error magnitudes of the ultrasound measures must take into account the use of different equipment and also the operator experience. Questions like the probe type, its orientation, level of compression caused by the probe, size of the measurement region, and ROI size and location are issues that must be considered to maximising the quality and accuracy of ultrasound imaging. New image processing tools must be developed to inform the operator that the conditions are maintained in different examinations.

In this thesis, we obtained a detailed morphologic characterisation of the four heads of the quadriceps muscle, using always the same ultrasound parameters, contributing for the understanding of muscle adaptations. However, it will be important to clarify if it is necessary to stablish different ultrasound settings for each muscle, namely the depth and probe frequency, to ensure that the "true" texture or EI of each muscle is

registered. Also, guidelines regarding anatomic landmarks should be developed as a means to enhance comparability between studies.

The interpretation of US imaging data requires further information. In strength training studies, the use of electromyography could be important in assessing or controlling the level of activity of the different muscles during the training sessions and the data could be compared with the ultrasound-derived parameters of muscle adaptation. The use of methods for assessing neuromuscular activity is needed to confirm the validity and feasibility of quantitative ultrasound for studying muscle function.

5.3 References

Agyapong-Badu, S., Warner, M., Samuel, D., Narici, M., Cooper, C., & Stokes, M. (2014). Anterior thigh composition measured using ultrasound imaging to quantify relative thickness of muscle and non-contractile tissue: a potential biomarker for musculoskeletal health. *Physiological Measurement*, *35*(10), 2165–76.

Bartlett, J., & Frost, C. (2008). Reliability, repeatability and reproducibility : analysis of measurement errors in continuous variables, (February), 466–475.

Blazevich, A., Gill, N., & Zhou, S. (2006). Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *Journal of Anatomy*, *209*(3), 289–310.

Brandenburg, J., Eby, S., Song, P., Zhao, H., Brault, J., Chen, S., & An, K. (2014). Ultrasound elastography: The new frontier in direct measurement of muscle stiffness. *Archives of Physical Medicine and Rehabilitation*, *95*(11), 2207–2219.

Branderburg, J., & Docherty, D.(2006). The effect of training volume on the acute response and adaptation to resistance training. *Int. J Sports Physiol. and Performance*, *1*, 108–121.

Cadore, E., Pinto, R., Bottaro, M., & Izquierdo, M. (2014). Strength and Endurance Training Prescription in Healthy and Frail Elderly. *Aging and Disease*, *5*(1), 1–13.

Caresio, C., Molinari, F., Emanuel, G., & Minetto, M. (2015). Muscle echo intensity: Reliability and conditioning factors. *Clinical Physiology and Functional Imaging*, *September*(5), 1–11.

Chino, K., Akagi, R., Dohi, M., Fukashiro, S., & Takahashi, H. (2012). Reliability and Validity of Quantifying Absolute Muscle Hardness Using Ultrasound Elastography, *7*(9), 5–9.

Cruz-Montecinos, C., Guajardo-Rojas, C., Montt, E., Contreras-Briceño, F., Torres-Castro, R., Díaz, O., & Cuesta-Vargas, A. (2016). Sonographic Measurement of the Quadriceps Muscle in Patients With Chronic Obstructive Pulmonary Disease. *Journal of Ultrasound in Medicine*, *35*(11), 2405–2412.

Boer, M., Seynnes, O., Prampero, P., Pisot, R., Mekjavić, I., Biolo, G., & Narici, M. (2008). Effect of 5 weeks horizontal bed rest on human muscle thickness and architecture of weight bearing and non-weight bearing muscles. *European Journal of Applied Physiology*, *104*(2), 401–7.

Drakonaki, E., Allen, G., & Wilson, D. (2012). Ultrasound elastography for musculoskeletal applications. *The British Journal of Radiology*, *85*(1019), 1435–45.

English, C., Fisher, L., & Thoirs, K. (2012). Reliability of real-time ultrasound for measuring skeletal muscle size in human limbs in vivo: a systematic review. *Clinical Rehabilitation*, *26*(10), 934–944.

Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., … Ichihashi, N. (2012). Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *European Journal of Applied Physiology*, *112*(4), 1519–25.

Harris-Love, M., Seamon, B., Teixeira, C., & Ismail, C. (2016). Ultrasound estimates of muscle quality in older adults: reliability and comparison of Photoshop and ImageJ for the grayscale analysis of muscle echogenicity. *PeerJ*, *4*, e1721.

Herrick, I., Brown, S., Agyapong-Badu, S., Warner, M., Ewings, S., Samuel, D., & Stokes, M. (2017). Anterior Thigh Tissue Thickness Measured Using Ultrasound Imaging in Older Recreational Female Golfers and Sedentary Controls. *Geriatrics*, *2*(1), 10.

Hirono, J., Mukai, N., Takayanagi, S., & Miyakawa, S. (2016). Changes in the hardness of the gastrocnemius muscle during a Kendo training camp as determined using ultrasound real-time tissue elastography. *The Journal of Physical Fitness and Sports Medicine*, *5*(3), 239–245.

Hug, F., Lacourpaille, L., Maïsetti, O., & Nordez, A. (2013). Slack length of gastrocnemius

medialis and Achilles tendon occurs at different ankle angles. *Journal of Biomechanics*, *46*(14), 2534–8.

Jansen, M., Alfen, N., Sanden, M., Dijk, J., Pillen, S., & Groot, I. (2012). Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. *Neuromuscular Disorders*, *22*(4), 306–317.

Jenkins, N.; Miller, J.; Buckner, S.; Cochrante, K.; Bergstrom, H.; Hill, E.; Smith, C.; Housh, T.; Cramer, J. (2015). Test–retest reliability of single transverse versus panoramic ultrasound imaging for muscle size and echo intensity of the biceps brachii. *Ultrasound in Medicine and Biology*, *41*(6), 1584–1591.

Jhu, J., Chai, H., Jan, M., Wang, C., Shau, Y., & Wang, S. (2010). Reliability and Relationship Between 2 Measurements of Transversus Abdominis Dimension Taken During an Abdominal Drawing-in Maneuver Using a Novel Approach of Ultrasound Imaging. *Journal of Orthopaedic & Sports Physical Therapy*, *40*(12), 826–832.

Konig, N., Cassel, M., Intziegianni, K., & Mayer, F. (2014). Inter-Rater Reliability and Measurement Error of Sonographic Muscle Architecture Assessments. *Journal of Ultrasound in Medicine*, *33*(5), 769–777.

Koo, T. , Guo, J., Cohen, J. , & Parker, K. (2014). Quantifying the passive stretching response of human tibialis anterior muscle using shear wave elastography. *Clinical Biomechanics (Bristol, Avon)*, *29*(1), 33–9.

Koppenhaver, S., Hebert, J., Fritz, J., Parent, E., Teyhen, D., & Magel, J. (2009). Reliability of rehabilitative ultrasound imaging of the transversus abdominis and lumbar multifidus muscles. *Archives of Physical Medicine and Rehabilitation*, *90*(1), 87–94.

Le Sant, G., Ates, F., Brasseur, J., & Nordez, A. (2015). Elastography study of hamstring behaviors during passive stretching. *PLoS ONE*, *10*(9), 1–13.

Levinson, S., Shinagawa, M., & Sato, T. (1995). Sonoelastic determination of human skeletal-muscle elasticity. *Journal of Biomechanics*, *28*(10), 1145–54.

Mangine, G., Hoffman, J., Gonzalez, A., Jajtner, A., Scanlon, T., Rogowski, J., … Stout, J. R. (2014). Bilateral Differences in Muscle Architecture and Increased Rate of Injury in National Basketball Association Players. *Journal of Athletic Training*, *49*(6), 794–799.

Muraki, T., Ishikawa, H., & Morise, S. (2014). Ultrasound elastography – based assessment of the elasticity of the supraspinatus muscle and tendon during muscle contraction. *Journal of Shoulder and Elbow Surgery*, 1–7.

Niitsu, M., Michizaki, A., Endo, A., Takei, H., & Yanagisawa, O. (2011). Muscle hardness measurement by using ultrasound elastography: a feasibility study. *Acta Radiologica (Stockholm, Sweden : 1987)*, *52*(1), 99–105.

Nordez, A., Gennisson, J., Casari, P., Catheline, S., & Cornu, C. (2008). Characterization of muscle belly elastic properties during passive stretching using transient elastography. *Journal of Biomechanics*, *41*(10), 2305–2311.

Palmer, T., Akehi, K., Thiele, R., Smith, D., & Thompson, B. (2015). Reliability of Panoramic Ultrasound Imaging in Simultaneously Examining Muscle Size and Quality of the Hamstring Muscles in Young, Healthy Males and Females. *Ultrasound in Medicine & Biology*, *41*(3), 675–684.

Pillen, S., Keimpema, M., Nievelstein, R., Verrips, A., Kruijsbergen-Raijmann, W., & Zwarts, M. (2006). Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound in Medicine and Biology*, *32*(9), 1315–1321.

Pillen, S., Tak, R., Zwarts, M., Lammens, M., Verrijp, K., Arts, I., … Verrips, A. (2009). Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound in Medicine & Biology*, *35*(3), 443–6.

Radaelli, R., Bottaro, M., Wilhelm, E., Wagner, D., & Pinto, R. (2012). Time Course of Strength and Echo Intensity Recovery After Resistance Exercise in Women. *Journal of Strength and Conditioning Research*, *26*(9), 2577–2584.

Raj, I., Bird, S., & Shield, A. (2012). Reliability of ultrasonographic measurement of the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults. *Clinical Physiology and Functional Imaging*, *32*(1), 65–70.

Ruas, C., Pinto, R., Lima, C., Costa, P., & Brown, L. (2017). Test-Retest Reliability of Muscle Thickness, Echo-Intensity and Cross Sectional Area of Quadriceps and Hamstrings Muscle Groups Using B-mode Ultrasound. *International Journal of Kinesiology and Sports Science*, *5*(1), 35.

Strasser, E., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–88.

Strasser, E., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–2388.

Varanoske, A., Fukuda, D., Boone, C. H., Beyer, K., Stout, J., & Hoffman, J. (2017). Homogeneity of echo intensity values in transverse ultrasound images. *Muscle & Nerve*, 19–21.

Verhulst, F., Leeuwesteijn, A., Louwerens, J., Geurts, A., Van Alfen, N., & Pillen, S. (2011). Quantitative ultrasound of lower leg and foot muscles: feasibility and reference values. *Foot and Ankle Surgery*, *17*(3), 145–9.

Wilhelm, E., Rech, A., Minozzo, F., Radaelli, R., Botton, C., & Pinto, R. (2014). Relationship between quadriceps femoris echo intensity, muscle power, and functional capacity of older men. *Age*, *4*(Fev 11), 1–6.

Yanagisawa, O., Niitsu, M., Kurihara, T., & Fukubayashi, T. (2011). Evaluation of human muscle hardness after dynamic exercise with ultrasound real-time tissue elastography: A feasibility study. *Clinical Radiology*, *66*(9), 815–819.

Attachements I – Ethical approval

FACULDADE DE MOTRICIDADE HUMANA

Conselho de Ética

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Paulo Armada (Vice-presidente)
Analiza Silva
Ana Rodrigues
Augusto Gil Pascoal
Margarida Matos
Paula Marta Bruno
Celeste Simões (suplente)
Herminio Barreto (suplente)

Para:
Dra. Rute Santos
Faculdade de Motricidade Humana

Data: 13 de maio de 2015

Projeto: *Reprodutibilidade dos Parâmetros Morfológicos (Eco-intensidade e Espessura) dos Músculos do Quadricípite Medidos por Ultrassonografia*

Este Conselho procedeu à apreciação de natureza ética do projeto em epígrafe de acordo com os procedimentos definidos nos seus Estatutos. Como resultado desta apreciação, considera-se que são necessárias informações adicionais ou a reformulação do projeto (tal como submetido ao CEFMH), previamente à emissão de um parecer definitivo por parte do CEFMH.

Em anexo seguem as considerações a dar resposta pelo proponente. Para este efeito, recomenda-se a consulta atenta do *Documento Orientador para Proponentes e Revisores*, disponível em anexo e na página web do CEFMH.

O projeto reformulado ou informações adicionais devem ser enviados para o email etica@fmh.ulisboa.pt, dirigidos ao Presidente do CEFMH, no prazo de quatro semanas desde a data desta carta.

O Presidente do Conselho de Ética da FMH

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Attachements II – Ethical approval II

Faculty of Human Kinetics
TECHNICAL UNIVERSITY OF LISBON

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Celeste Simões (supl.)
Gonçalo Tavares (supl.)

To: Dr. Maria João Valamatos
Faculdade de Motricidade Humana

Date: July 9, 2013

Research Project: *Arquitectura Muscular e Treino de Força. Influência do Tipo de Acção Muscular e da Amplitude de Movimento*

This Council has reviewed the project indicated above. Based on independent reviews by two experts of all information provided by the proponent, we declare that this project is in accordance with Portuguese and international guidelines for scientific research involving human beings, including the 2008 Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, and the 1997 Convention on Human Rights and Biomedicine (the "Oviedo Convention").

The President of the Ethics Council

Pedro J. Teixeira, Ph.D.

Ethics Council of the Faculty of Human Kinetics, Technical University of Lisboa
Conselho de Ética da Faculdade de Motricidade Humana, Universidade Técnica de Lisboa
Faculdade de Motricidade Humana
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Attachements III – Informed Consent I

FACULDADE DE MOTRICIDADE HUMANA

LISBOA
UNIVERSIDADE DE LISBOA

Consentimento Informado, Livre e Esclarecido

Tomei conhecimento de toda a informação referente a este estudo, nomeadamente os objectivos, os procedimentos e benefícios e riscos inerentes aos mesmos. Fui ainda informado que este meu contributo não envolve quaisquer encargos para mim, exceto eventualmente os decorrentes do transporte até ao local do estudo. Além disso, foi-me afirmado que tenho o direito a recusar a todo o tempo a participação no estudo, sem que isso possa ter como efeito qualquer prejuízo pessoal ou profissional. Foi-me dada oportunidade de fazer as perguntas que julguei necessárias, e de todas obtive resposta satisfatória. Os registos dos resultados poderão ser consultados pelos responsáveis científicos e ser objecto de publicação, mas os elementos da identidade pessoal serão sempre tratados de modo estritamente confidencial.

Declaro que li o presente documento e estou consciente do que esperar quanto à minha participação no estudo *Reprodutibilidade dos parâmetros morfológicos (eco-intensidade e espessura) dos músculos do quadricípite, por ultrassonografia*. Assim, aceito voluntariamente participar neste estudo. Ser-me-á fornecida uma cópia deste documento.

Nome do participante **Assinatura do participante**

Data

Nome do representante legal do participante
(se aplicável)

Grau de relação com o participante

Investigador/Equipa de Investigação

Os aspetos mais importantes deste estudo foram explicados ao participante ou ao seu representante, antes de solicitar a sua assinatura. Uma cópia deste documento ser-lhe-á fornecida.

Nome da pessoa que obtém o consentimento **Assinatura da pessoa que obtém o consentimento**

Data

Attachements IV – Informed Consent II

FACULDADE DE MOTRICIDADE HUMANA

LISBOA
UNIVERSIDADE DE LISBOA

Consentimento Informado, Livre e Esclarecido

Tomei conhecimento de toda a informação referente a este estudo, nomeadamente os objectivos, os procedimentos e benefícios e riscos inerentes aos mesmos. Fui ainda informado que este meu contributo não envolve quaisquer encargos para mim, exceto eventualmente os decorrentes do transporte até ao local do estudo. Além disso, foi-me afirmado que tenho o direito a recusar a todo o tempo a participação no estudo, sem que isso possa ter como efeito qualquer prejuízo pessoal ou profissional. Foi-me dada oportunidade de fazer as perguntas que julguei necessárias, e de todas obtive resposta satisfatória. Os registos dos resultados poderão ser consultados pelos responsáveis científicos e ser objecto de publicação, mas os elementos da identidade pessoal serão sempre tratados de modo estritamente confidencial.

Declaro que li o presente documento e estou consciente do que esperar quanto à minha participação no estudo *Avaliação da rigidez muscular por elastografia de cisalhamento*. Assim, aceito voluntariamente participar neste estudo. Ser-me-á fornecida uma cópia deste documento.

Nome do participante **Assinatura do participante**

Data

Nome do representante legal do participante
(se aplicável)

Grau de relação com o participante

Investigador/Equipa de Investigação

Os aspetos mais importantes deste estudo foram explicados ao participante ou ao seu representante, antes de solicitar a sua assinatura. Uma cópia deste documento ser-lhe-á fornecida.

Nome da pessoa que obtém o consentimento **Assinatura da pessoa que obtém o consentimento**

Data