Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa de Engenharia
COPPE UFRJ

PATELLAR TENDON MECHANICAL PROPERTIES ADAPTATION TO A RESISTANCE TRAINING PROTOCOL MEASURED BY ELASTOGRAPHY

Pietro Mannarino

Tese de Doutorado apresentada ao Programa de Pós-graduação em Engenharia Biomédica, COPPE, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor em Engenharia Biomédica.

Orientadora: Liliam Fernandes de Oliveira

Rio de Janeiro
Junho de 2019

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TESE SUBMETIDA AO CORPO DOCENTE DO INSTITUTO ALBERTO LUIZ COIMBRA DE PÓS-GRADUAÇÃO E PESQUISA DE ENGENHARIA (COPPE) DA UNIVERSIDADE FEDERAL DO RIO DE JANEIRO COMO PARTE DOS REQUISITOS NECESSÁRIOS PARA A OBTENÇÃO DO GRAU DE DOUTOR EM CIÊNCIAS EM ENGENHARIA BIOMÉDICA.

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RIO DE JANEIRO, RJ - BRASIL

JUNHO DE 2019

Mannarino, Pietro

Patellar Tendon Mechanical Properties Adaptation to a Resistance Training Protocol Measured by Elastography/ Pietro Mannarino. – Rio de Janeiro: UFRJ/COPPE, 2019.

XIII, 75 p.: il.; 29,7 cm.

Orientador: Liliam Fernandes de Oliveira

Tese (doutorado) – UFRJ/ COPPE/ Programa de Engenharia Biomédica, 2019.

Referências Bibliográficas: p. 43-53

1. Propriedades mecânicas. 2. Tendão patelar. 3. Treinamento resistido. I. Oliveira, Liliam Fernandes de. II. Universidade Federal do Rio de Janeiro, COPPE, Programa de Engenharia Biomédica. III. Título.

DEDICATÓRIA

Aos meus filhos Luciano e Renato,

sentido de tudo que faço.

AGRADECIMENTOS

Agradeço à minha esposa Ana Carolina, por todo o esforço e amor dedicados, sem os quais eu não “andaria” tão longe.

Agradeço aos meus pais Márcia e Luciano, por semearem em mim a empatia pelo conhecimento.

Agradeço ao eterno mestre César Fontenelle, por toda amizade, cooperação e crença no meu potencial.

Agradeço à Professora Liliam Fernandes de Oliveira, por todos os ensinamentos e pelo exemplo a ser seguido.

Agradeço aos incansáveis Maria Clara Brandão e Lino Matias, por toda a cooperação e entrega desinteressada.

Finalmente, agradeço a Deus, por ter colocado todas essas pessoas na minha vida.

EPÍGRAFE

"Daria tudo que sei pela metade do que ignoro"

- René Descartes

Resumo da Tese apresentada à COPPE/UFRJ como parte dos requisitos necessários para a obtenção do grau de Doutor em Ciências (D.Sc.)

ADAPTAÇÃO DAS PROPRIEDADES MECÂNICAS DO TENDÃO PATELAR A UM PROGRAMA DE TREINAMENTO RESISTIDO MEDIDAS PELA ELASTOGRAFIA

Pietro Mannarino

Junho de 2019

Orientadora: Liliam Fernandes de Oliveira

Programa: Engenharia Biomédica

O tendão patelar é um dos mais relevantes do corpo humano por sua importância fundamental na marcha e ortostatismo. Suas propriedades mecânicas estão intimamente relacionadas à função do quadríceps, sua sobrecarga habitual e aos programas de treinamento ao qual é submetido. Este trabalho avalia a adaptação do módulo de cisalhamento do tendão patelar a um programa de treinamento resistido de oito semanas através do uso da elastografia supersonicshearwaveimaging (SSI), em 15 homens jovens e saudáveis. Além disso, foram mensuradas as variações do módulo de cisalhamento e espessura do vasto lateral do quadríceps, as adaptações morfológicas do tendão patelar e o aumento do torque extensor do joelho utilizando o ultrassom modo B e dinamometria. Os resultados observados atestam a eficiência do treinamento aplicado em promover aumento de força e hipertrofia no músculo alvo, porém não revelaram adaptações estatisticamente significantes no módulo de cisalhamento ou espessura do tendão patelar. Foi observada uma elevação pronunciada e estatisticamente significante no módulo de cisalhamento do vasto lateral. Esse estudo pode servir de base para ulteriores investigações sobre protocolos de intervenção que visam adaptar o mecanismo extensor do joelho para prevenção de lesões, reabilitação ou condicionamento.

Abstract of Thesis presented to COPPE/UFRJ as a partial fulfillment of the requirements for the degree of Doctor of Science (D.Sc.)

PATELLAR TENDON MECHANICAL PROPERTIES ADAPTATION TO A RESISTANCE TRAINING PROTOCOL MEASURED BY ELASTOGRAPHY

Pietro Mannarino

Junho de 2019

Orientadora: Liliam Fernandes de Oliveira

Department: Biomedical Engineering

The patellar tendon is one of the most relevant in the human body because of its fundamental importance in gait and orthostatism. Its mechanical properties are closely related to quadriceps function, its usual overload and training programs to which it is subjected. This study evaluates the adaptation of the patellar tendon´s shear modulus to an 8-week resistance training program, using supersonic shear wave imaging (SSI) elastography in 15 healthy young men. In addition, the variations of the quadriceps´ vastus lateralis´ shear modulus and thickness, the morphological adaptations of the patellar tendon and the increase of knee extensor torque were measured using B-mode ultrasound and dynamometry. The observed results attest the efficiency of the resistance training program applied to promote strength gains and hypertrophy, but did not reveal statistically significant adaptations in the shear modulus or thickness of the patellar tendon. A pronounced and statistically significant elevation was observed in the vastus lateralis´ shear modulus. This study may serve as a basis for further investigations into intervention protocols aimed to adapt the knee extensor mechanism for injury prevention, rehabilitation or conditioning.

TABLES

FIGURE LIST

ABBREVIATION LIST:

CI – confidence interval

CSA – cross sectional area

E – Young´s Modulus

KE – knee extension

KT – knee extension torque

MT – muscle thickness

PT – patellar tendon

PTT – patellar tendon thickness

RM – repetitium maximum

ROI – region of interest

RI – rest interval

RT – resistance training

SD – standard deviation

SQ – squat

SSI – supersonic shearwave imaging

SWE – shear wave elastography

US – ultrasound

VL – vastus lateralis

μ – shear modulus

λ – Lamé modulus

1. INTRODUCTION

Skeletal muscles act as active units and primary motors for the body segments movements (LIEBER *et al.*, 2017), while tendons represent an important connective tissue with high resistance to tensile loading, responsible for muscle force transmission to the bone (BENJAMIN *et al.*, 2006). Both tendon tensile environment and muscle demand levels will determine adaptations in these structures (COUPPE *et al.*, 2008; KUBO; KANEHISA; FUKUNAGA, 2002). According to the overload environment, tendons can increase resistance, stiffness and thickness or undergo inflammation and structural disorganization (GALLOWAY; LALLEY; SHEARN, 2013).Increased muscle demand and training regime, especially against an high external resistance, can result in hypertrophy, changes in architecture or, sometimes, lesions and degeneration (AMERICAN COLLEGE OF SPORTS MEDICINE, 2009; LIEBER *et al.*, 2017).

Due to its fundamental function for standing in upright position, walking and running and significant impairment in normal movement during pathologic situations, knee extensor mechanism (quadriceps and patellar tendon (PT)) mechanical properties have always received particular interest (COUPPÉ *et al.*, 2013; GROSSET *et al.*, 2014; KOT *et al.*, 2012; PEARSON; BURGESS; ONAMBELE, 2007; ZHANG, Z. J.; NG; FU, 2015). Also, the PT deserves special attention due to its peculiar anatomy and high incidence of injuries, specially the one known as “jumper´s knee”(ZHANG, Z. J.; NG; FU, 2015; ZHANG, ZHI JIE *et al.*, 2014).

In the last decades, changes in PT mechanical properties have been object of many studies. Its adaptations to overload regimen, resistance training (RT) protocols, ageing and pathologic conditions were extensively documented (CARROLL *et al.*, 2008; COUPPÉ *et al.*, 2013; O’BRIEN *et al.*, 2010; TAŞ *et al.*, 2017). Yet, the PT structural and histological adaptations to overloading are still not fully understood. Much of the knowledge about its mechanical adaptations*in vivo*is derived from indirect analysis based on B-mode (Bright mode) ultrasound (US) imaging combined with dynamometry.

Using determined anatomical landmarks and a known external resistance, it is possible to calculate some tendon mechanical properties, especially Young´s modulus (E) and stiffness. This method, however, is subject to inaccuracies due to possible calibration errors and misinterpretations of the structures displacement (BURGESS *et al.*, 2009; PEARSON; BURGESS; ONAMBELE, 2007).

Due to the close interactionbetween tendons and muscles´ connective tissue with the contractile elements of skeletal muscle, it is relevantto investigate the mechanicsof the relatedmuscle when studying tendons´ behavior to loading. Muscle mechanical properties analysis is not feasible using this strategy due the dynamic nature of muscle contraction, even in isometric actions, and the absence of intrinsic anatomical landmarks to calculate strain and displacement(LIEBER *et al.*, 2017; YANAGISAWA, O. *et al.*, 2011). Other methodologies, as an application of a durometer to describe a muscle hardness index are also very limited and the reproducibility of this specific device has not been systematically evaluated (NIITSU *et al.*, 2011).

In that context, Shear Wave Elastography (SWE) received particular interest in US imaging routine, allowing a static evaluation of tissues mechanical properties (AKAGI *et al.*, 2016; KOT *et al.*, 2012). Supersonic Shearwave Imaging (SSI), a recent evolution of conventional SWE, is able to determine the shear modulus (µ) in a determined region of interest (ROI) based on the combination of a radiation force and an ultrafast acquisition imaging system (BERCOFF; TANTER; FINK, 2004; LIMA *et al.*, 2017; ZHANG, ZHI JIE *et al.*, 2014). The µ is therefore computed from the velocity of the propagating shear waves assuming an approximate isotropic and homogeneous medium, allowing real time analysis (BRUM *et al.*, 2014; ROYER *et al.*, 2011).

Analyzing the PT, SWE has revealed PT µ reducing after stretch-shortening regimens(ZHANG, Z. J.; NG; FU, 2015), ageing(HSIAO *et al.*, 2015), tendinopathy(OOI, CHIN CHIN *et al.*, 2016; ZHANG, ZHI JIE *et al.*, 2014) and surgical procedures (BOTANLIOGLU *et al.*, 2016). SWE was also externally validated and although µ and tensile elastic modulus represent different measures, PT µ showed a strong positive correlation to longitudinal Young´s Modulus (E), ultimate force to failure and resistance to tensile loading in *in vitro* models (MARTIN *et al.*, 2015; YEH *et al.*, 2016).

In muscle study, SWE has showed a strong positive correlation to the muscle loading environment passively or during active contraction ($R^2 > 0.9$) (ATEŞ *et al.*, 2015; BOUILLARD, K. *et al.*, 2012; BOUILLARD, KILLIAN; NORDEZ; HUG, 2011). SWE also detected reductions in muscle µ after long term stretching protocols (AKAGI; TAKAHASHI, 2014), eccentric resistance training (SEYMORE *et al.*, 2017), aging (AKAGI; YAMASHITA; UEYASU, 2015) and musculoskeletal pathologies (BOTANLIOGLU *et al.*, 2013).In the other hand, increases in muscle µ were registered in acute muscle damage (AKAGI *et al.*, 2015).

Although PT mechanical adaptations and quadriceps structural changes after resistance training monitored by US are extensively documented (KRAEMER *et al.*, 2009; WIESINGER *et al.*, 2015), to our knowledge no previous studies evaluated the PT or quadriceps µ changes promoted by a RT protocol using SWE. These changes in tendon collagen cross-linking andcomposition promoted by RT strategies that can impact ultimate force to failure and resistance to injury, can be detected by SSI(MARTIN *et al.*, 2015; YEH *et al.*, 2016). Therefore, this tool could be useful to analyze variations of tendon mechanical properties reflected by the μ and helpdesigning optimal strategies based on RT for injury prevention and rehabilitation.

Therefore, the objective of this study was to use the SWE to analyze the PT mechanical properties response to an 8-week RT protocol. Also, SWE was used to study the quadriceps vastus lateralis (VL) response to the intervention. Furthermore, we tested the effectiveness of the RT protocol by evaluating the knee extensor mechanism gains of strength and hypertrophy by measuring the knee extensor torque (KT) and the vastus lateralis muscle thickness (VL MT) pre and post intervention. Lastly, we studied the reliability of the SSI evaluation and the correlation between changes in the PT μ and the oscillation of all the variables tested pre and post intervention.

2.1. LITERATURE REVIEW

To provide greater insight on this research, a brief literature review was conducted. In this topic will be approached the material properties and mechanobiology of tendons, the SSI basic science and evolution, the tendons development, morphogenesis and adaptation process, the evolution of the study of patellar tendon properties and the patellar tendon adaptations to overloading and resistance training.

2.1.1. Material properties and mechanobiology of tendons

The mechanical behavior of a given material or structure to loading is intimately related to its mechanical properties, namely elasticity and viscosity, and its dimensions, such as cross-sectional area and length. Stress (σ) applied to a given object will be proportional the force (F) applied to its initial area (A_0) (Eq. 1) and the strain (ε) will depend on the variation from the initial length (l_0) (Eq. 2).

$$\sigma = \frac{F}{A_0} \tag{1}$$

$$\varepsilon = \frac{l - l_0}{l_0} \tag{2}$$

Elasticity (k) is defined as a ratio between force and displacement (x) (Hooke´s Law, Eq. 3a)

$$k = \frac{F}{x} \tag{3a}$$

where k is a constant known as the rate or spring constant. It can also be stated as a relationship between stress and strain (Eq. 3b).

$$E = \frac{\sigma}{\varepsilon} \tag{3b}$$

where E is known as the elastic modulus or Young´s modulus. Viscosity is defined as the ratio of shearing stress to velocity gradient (Newton´s law of viscosity, Eq. 4).

$$\sigma(t) = \eta \frac{de(t)}{dt} \tag{4}$$

The main viscoelastic models - Maxwell, Voigt/Kelvin and Standard Linear Solid - represent viscosity and elasticity as spring and dashpot models (Fig. 1) and are useful as models for biological structures. The combination of viscous and elastic properties in tendons will determine its response to loading.

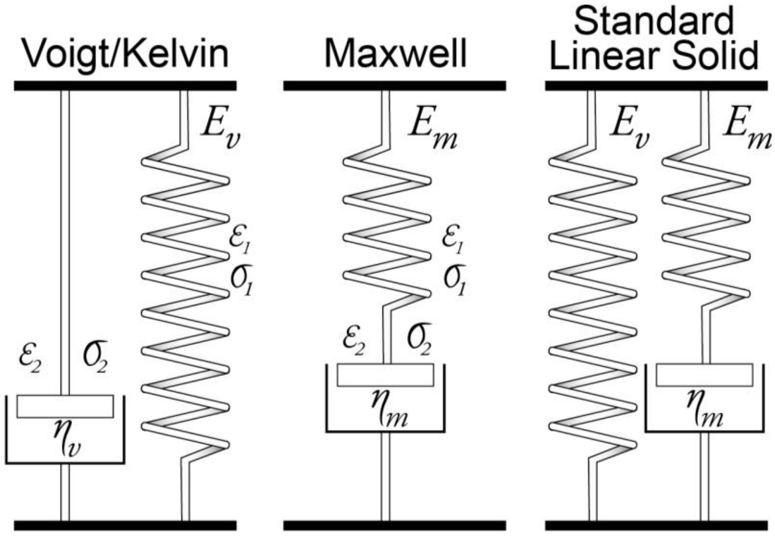

Figure 1. Spring-dashpot classic model for viscoelastic representation.

Tendons exhibit a hybrid behavior namely viscoelastic. This particular pattern creates another variable of great interest. The area exhibited as delay between loading and unloading will represent energy lost or dissipated known as hysteresis (Fig. 2).

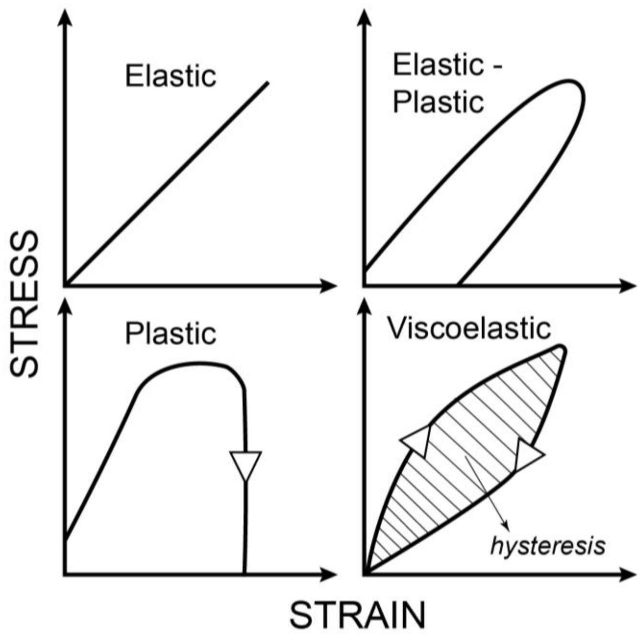

Figure 2. Stress-strain curves illustrating different types of behavior. Hysteresis represented as the area between loading and unloading in viscoelastic patterns.

When a given structure receives a tensile load in its longitudinal axis, a longitudinal stretching will take place as the same time as a transversal contraction. This will occur inversely during axial compression with transversal enlargement. The relation between linear strain and lateral strain is known as Poisson´s ratio (Fig. 3).

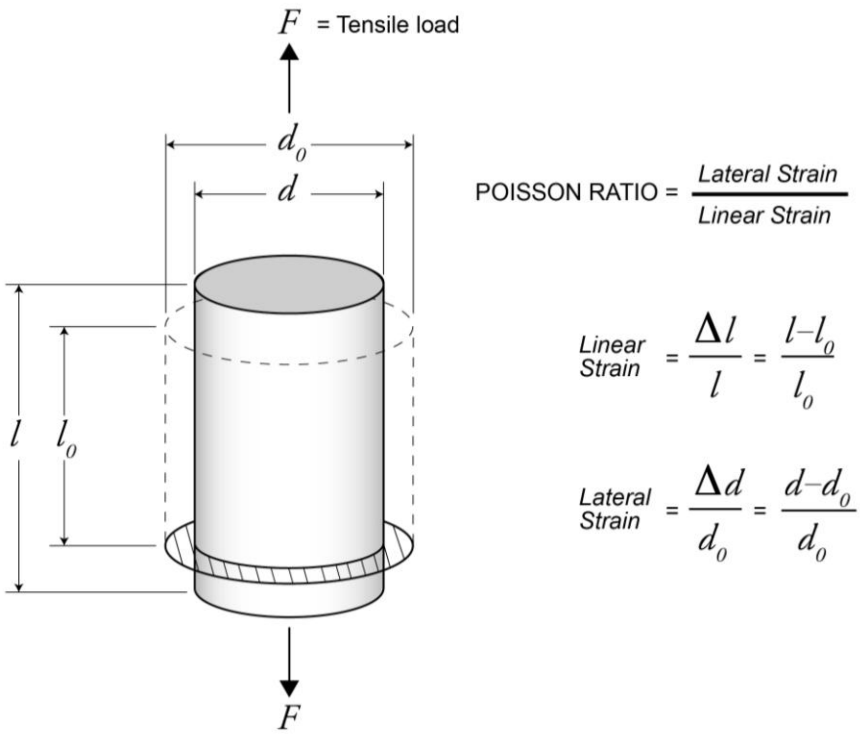

Figure 3. The Poisson´s ratio.

In a simplified analysis, tension and deformation are proportional and linked by the spring constant *k,* which represent a matrix of constants (Fig. 4).

$$\begin{pmatrix}\sigma_{xx}\\ \sigma_{yy}\\ \sigma_{zz}\\ \sigma_{yz}\\ \sigma_{zx}\\ \sigma_{xy}\end{pmatrix} = \begin{bmatrix}\lambda+2\mu & \lambda & \lambda & & & \\ \lambda & \lambda+2\mu & \lambda & & & \\ \lambda & \lambda & \lambda+2\mu & & & \\ & & & \mu & & \\ & & & & \mu & \\ & & & & & \mu\end{bmatrix} \cdot \begin{pmatrix}e_{xx}\\ e_{yy}\\ e_{zz}\\ e_{yz}\\ e_{zx}\\ e_{xy}\end{pmatrix}$$

Figure 4. Matrix of constants represented by the spring constant *k*.

Hooke´s law, in a purely elastic isotropic medium, can reduce the number of constants to two (Eq. 5).

$$\sigma_{ij}^{0} = \lambda\theta\delta_{ij} + 2\mu e_{ij} \qquad \text{i,j = 1,2,3...} \quad (5)$$

where λ and μ are Lamé constants and θ is the volume expansion.

Lamé´s first parameter μ is known as the shear modulus and given in equation 6a, while the second parameter λ is known as the Lamé modulus and calculated by equation 6b.

$$\mu = \frac{E}{2(1+v)} \qquad (6a)$$

$$\lambda = \frac{E}{(1+v)(1-2v)} \qquad (6b)$$

where E is the Young´s modulus and v is the Poisson´s ratio.

Young´s modulus can also be estimated in function of Lamé´s constants (Eq. 7a). Using Eq. 6b, we can estimate E in terms of μ and v (Eq.7b).

$$E = \mu \times \frac{(3\lambda+2\mu)}{\lambda+\mu} \qquad (7a)$$

$$E = \mu \times 2(1+v) \qquad (7b)$$

Equations 7a-b can be simplified to optimize Young's modulus parameter when it comes to biological tissues. Biological tissues were defined as the medium was considered to be almost incompressible and v was preset as 0.5(SARVAZYAN *et al.*, 1998). Second, in soft tissues, λ is 10^6 times greater than μ (Fig. 5), which allows us to suppress the constant 2μ and the denominator μ in Equation 7a(GENNISSON, J.-L. *et al.*, 2003).Applying these concepts, it is reasonable to state that the Young's modulus is three times the μ.

Equation 6a simplified: $E = \mu \times \frac{(3\lambda+\cancel{2\mu})}{\lambda+\cancel{\mu}} \therefore E = \frac{3\cancel{\lambda}\mu}{\cancel{\lambda}} \therefore \quad E \cong 3\mu$ (8)

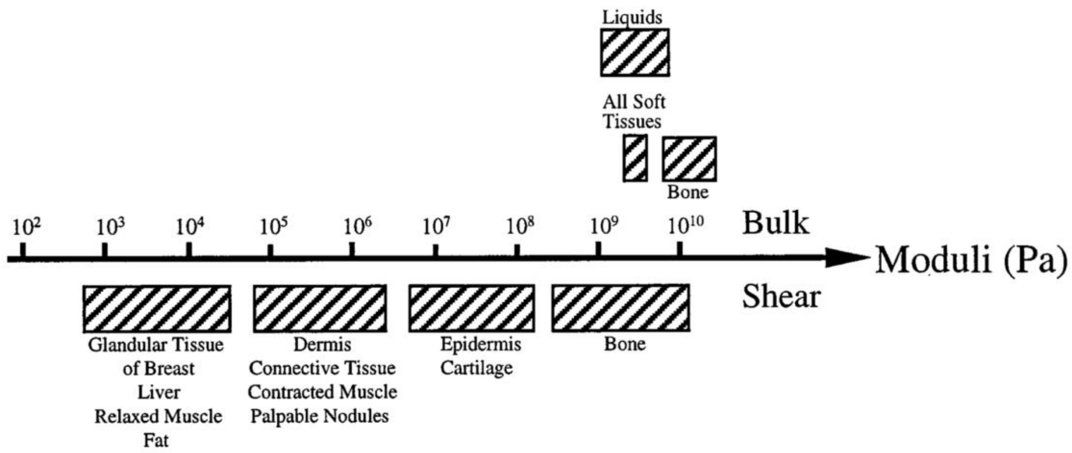

Figure 5. Different orders of magnitude for the volumetric (Bulk) modulus and shear modulus(adapted from SARVAZYAN *et al.*, 1998).

When analyzing pure isotropic mediums, stress-strain ratio exhibits a linear behavior (Fig.2, elastic). However, in biological tissues as tendons, stress-strain relation exhibits a non-linear behavior with four phases: toe region, elastic fase, plastic fase and failure (Fig. 6). This loading pattern and hysteresis makes tendons viscoelastic properties analysis much more complicated.

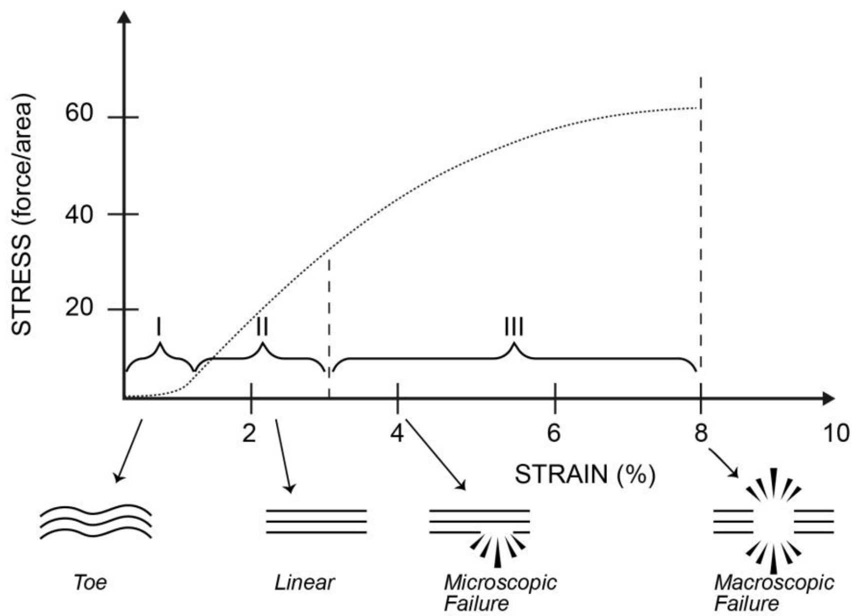

Figure 6. Tendons stress-strain behavior in different phases (adapted from YEH et al., 2016).

2.1.2. Tendon structure, morphogenesis and adaptation process

Tendons are composed essentially by collagen fibers highly organized hierarchically into fibrils, fibers and fascicles and other extra-cellular matrix (ECM) proteins. The main purpose of this special architecture is to withstand high tensile forces and guarantee force transmission (KJAER, 2004). Tendons consist of 55-70% water, mainly associated to proteoglycans in the ECM. From its dry weight, 60-85% is collagen, mostly type I (60%) (ELLIOTT, 1965; JOZSA; KANNUS, 1997; KER; ALEXANDER; BENNETT, 1988).

The tendon's hierarchical structure begins at the molecular level with tropocollagen. Approximately five tropocollagen molecules form a microfibril, which then aggregate to create a subfibril. Several subfibrils form a single fibril. Multiple fibrils form a tendon fascicle, and fascicles, separated by the endotenon, join to form the macroscopic tendon (Fig. 7). Tendon fibroblasts, or tenocytes, are found on collagen fibers allowing for the regulation of the extracellular environment in response to chemical and mechanical cues(GALLOWAY; LALLEY; SHEARN, 2013).

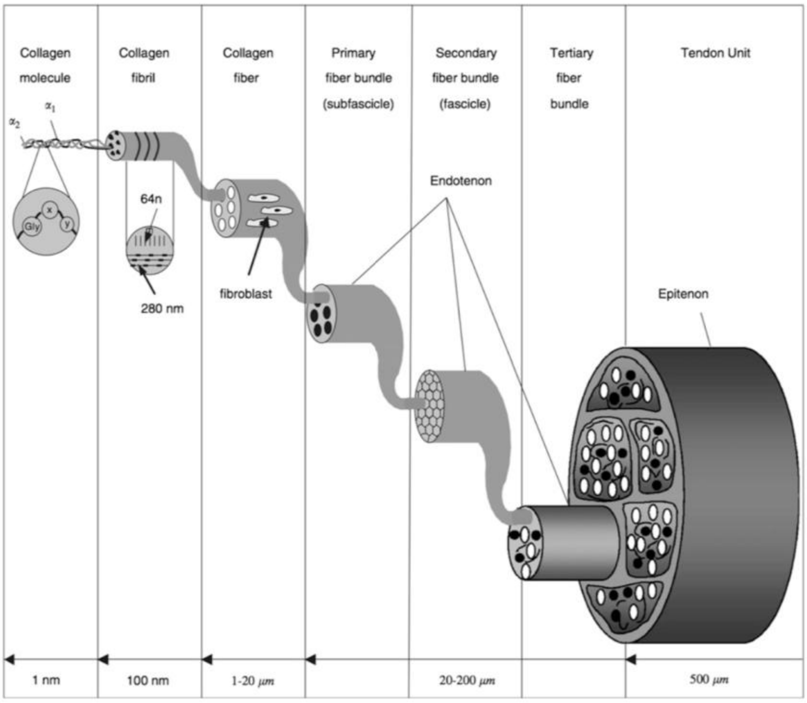

Figure 7. Tendon hierarchical structure (reproduced with permission from Elsevier from Silver et al. 2003).

Tendons´ collagen and ECM turnover can be modified by mechanical loading or inversely, stress shielding. Collagen synthesis, metalloproteases enzymes, transcription and posttranslational modifications as well as local and systemic growth factors are enhanced following exercise (KJAER, 2004). This will directly impact mechanical properties and viscoelastic characteristics of the tendon. Moreover, changes in CSA and collagen cross-linking will decrease the stress and increase ultimate resistance to failure. These processes are mainly deflagrated by mechanotransduction which triggers intracellular signaling. Secondarily, cell growth and survival (FU *et al.*, 2002; REID *et al.*, 1994; RUOSLAHTI, 1997; TIBBLES; WOODGETT, 1999), changes in morphology and architecture (CHICUREL; CHEN; INGBER, 1998; VANDENBURGH *et al.*, 1996) and also metabolic response (IHLEMANN *et al.*, 1999) will occur.

The main molecule responsible for adhesion between the cytoskeleton and the ECM are the Integrins (BURRIDGE; CHRZANOWSKA-WODNICKA, 1996; CHIQUET, 1999). They guarantee a mechanical connection which transmit forces from the outside to the inside of the cell, and vice versa (GOLDSCHMIDT; MCLEOD; TAYLOR, 2001; INGBER *et al.*, 1994; WANG, J. H. C., 2006; WANG, N. *et al.*, 2001). This “mechanical sensor” function was already attributed to the Integrins and it is believed that in conjunction with the cell cytoskeleton they function as a mechanical sensitive organelle (INGBER *et al.*, 1994). Mechanical transduction will deflagrate several signaling pathways, including focal adhesion kinase (FAK), integrin-linked kinase (ILK-1) and the most prominent mitogen-activated protein kinase (MAPK) (CHIQUET, 1999; FLUCK *et al.*, 1999; FLÜCK *et al.*, 2000; GOODYEAR *et al.*, 1996; TAKAHASHI *et al.*, 2003). MAPK is considered crucial producing transcriptions factors but also protein synthesis on translational level.

The tendons remodeling process mediated by overloading is also dependent of hormones and growth factors, each one associated to specific functions. TGF-α, IL-1, IL-6, IL-8, insulin growth factor I (IGF-I), fibroblast growth factor (FGF), nitric oxide (NO), prostaglandins, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), all have positive effects on fibroblast activation, which is the main responsible for collagen synthesis (CHIQUET, 1999; KJAER, 2004). Collagen synthesis is characterized not only by an increase in collagen amount but also by the presence of an extensive number of modifications of the polypeptide chains, which contribute to the quality and stability of the collagen molecule. Based on these findings it is widely accepted that RT increases collagen turnover and that collagen type I net synthesis enhancement is to be expected in the long term(LANGBERG, H. *et al.*, 1999; LANGBERG, HENNING; ROSENDAL; KJÆR, 2001).

It is crucial to understand that tendons and muscles connective tissue interact closely with the contractile elements of skeletal muscle. The tendon elasticity and stiffness will determine wherever it will act mainly as spring and buffer for energy absorption and storage or a rigid and quick force transmission cable. Both action patterns can be useful in activities with stretch-shortening cycles (high elasticity) or explosive force generation (high stiffness) (MALLIARAS *et al.*, 2013; TARDIOLI; MALLIARAS; MAFFULLI, 2012; ZHANG, Z. J.; NG; FU, 2015; ZHANG, ZHI JIE *et al.*, 2014). In both situations, CSA is known to decrease stress across the tendon and increase ultimate force to failure. Changes in tendon diameter secondary to progressive overload protocols or habitual chronic overloading conditions can be considered an adaptive and protective mechanism (COUPPE *et al.*, 2008).

When addressing specifically the RT situations, increase in tendon stiffness can be explained by the same twofold mechanisms hypothesized to explain tendon tissue maintenance (ARCHAMBAULT; HART; HERZOG, 2001; BIRCH, H L *et al.*, 1999). On one hand, an increase production of ECM proteins stemming from the mechanotransducive response of fibroblasts may serve to optimize force transmission and/or strengthen tendons (ARCHAMBAULT; HART; HERZOG, 2001). On the other hand, fatigue damage occurs at relatively low stress levels (BIRCH, HELEN L.; BAILEY; GOODSHIP, 1998) and routine remodeling and repair of the collagenous scaffold seem to be inherent features of tendons' design and maintenance. Either way, the integration of synthesized collagen into the tendon structure is poorly understood but this process may promote growth and/or a change in material properties (Fig. 8).

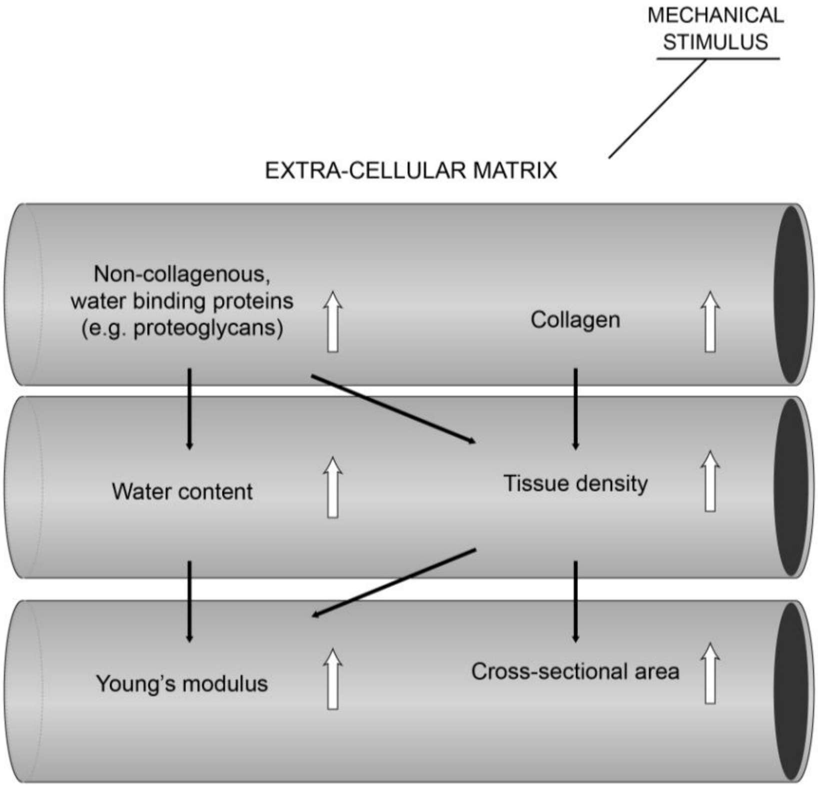

Figure 8. Theoretical model showing tendon material properties adaptation and hypertrophy to mechanical stimulus in resistance training (adapted from WIESINGER *et al.*, 2015).

Because tendon hypertrophy seems limited or insubstantial after short-term resistance training and because an increase in the E is almost systematically reported, the latter may constitute the main adaptive process by which the tendon becomes stiffer in the first weeks (Fig. 9) (WIESINGER *et al.*, 2015).

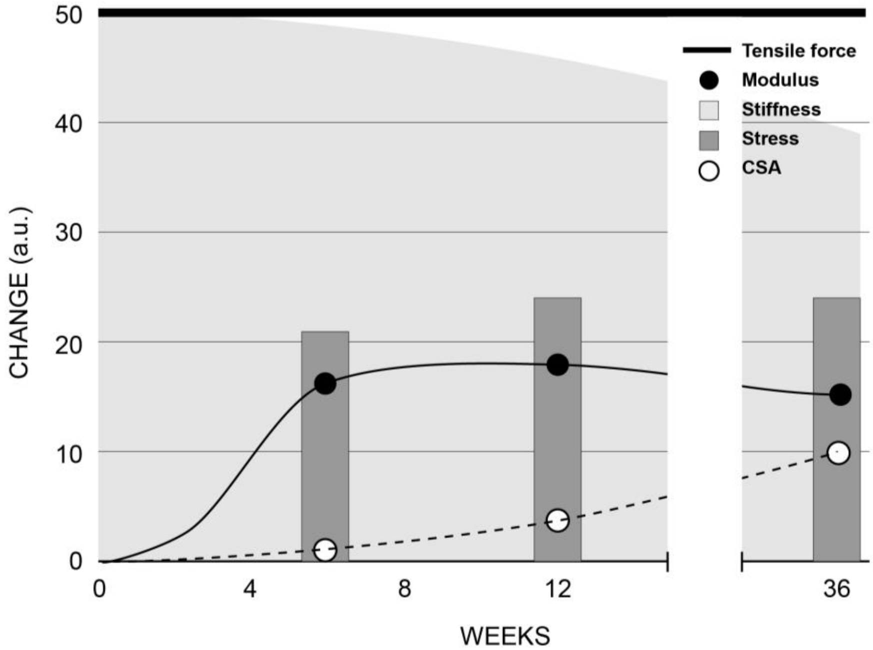

Figure 9. Hypothetical short- and long-term changes in tendon properties in response to constant overloading (adapted from WIESINGER *et al.*, 2015).

2.1.3. Evolution of the study of patellar tendon properties

The study of tendons viscoelastic properties has been of particular interest in the last decades. Initially, the assessment through calculation was made using ultrasonography (US) and magnetic resonance imaging (MRI) combined with dynamometry (HANSEN *et al.*, 2006; ONAMBELE; BURGESS; PEARSON, 2007; SEYNNES *et al.*, 2009, 2013; SVENSSON *et al.*, 2012).Most protocols consisted in applying a known tensile force to tendon through isometric contraction, measuring the consequent longitudinal deformation suffered(HANSEN *et al.*, 2006; HELLAND *et al.*, 2013). Knowing the deformation sustained and the force resisted, it was possible to calculate the tendon stiffness.

Some studies investigated the longitudinal E, calculated by the relation between stress (F/A) and strain (relative deformation) (COUPPE *et al.*, 2008). Obtaining this mechanical property demand the additional acquisition on tendon´s CSA through US or most accurately MRI. Although reproducible (HANSEN *et al.*, 2006), these strategies are time consuming, inaccurate (ONAMBELE; BURGESS; PEARSON, 2007), restricted to large hinge joints and unable to directly determine the tissue elastic properties (HSIAO *et al.*, 2015; OOI, C. C. *et al.*, 2014).

Although developed almost three decades ago (OPHIR *et al.*, 1991), only recently SWE was applied to directly determine PT elasticity *in vivo*(KOT *et al.*, 2012). Probably, SWE represents the most important technical development in the field of ultrasonography since Doppler imaging (DRAKONAKI; ALLEN; WILSON, 2012). Its real-time imaging can estimate in vivo tissue strain distribution; however, it relies on compressive force applied externally. This compressive force can be made manually (freehand elastography) (BERKO *et al.*, 2015; PORTA *et al.*, 2014)or mechanically (transient elastography), which can impair reproducibility (BERCOFF; TANTER; FINK, 2004). Also, external compression, especially when made manually without any pressure determination device, may alter mechanical properties of the testing structure (KOT *et al.*, 2012).

In that context, SSI rises as a valuable tool for PT evaluation (HSIAO *et al.*, 2015; KOT *et al.*, 2012; PELTZ *et al.*, 2013; ZHANG, Z. J.; NG; FU, 2015; ZHANG, ZHI JIE *et al.*, 2014; ZHANG, ZHI JIE; FU, 2013). It´s able to determine the elastic properties in a determined ROI without need of further calculus or estimations (JIANG *et al.*, 2015; KOT *et al.*, 2012), what is useful for different structures in large or small joints, in healthy or injury tendons (OOI, CHIN CHIN *et al.*, 2016; ZHANG, ZHI JIE *et al.*, 2014). Also there is no need for external compression, which turns this technique probably more reproducible and less operator-dependent (HSIAO *et al.*, 2015; KOT *et al.*, 2012; PELTZ *et al.*, 2013; ZHANG, ZHI JIE; FU, 2013).

SSI uses acoustic radiation force created by ultrasound to perturb tissues and create a wave propagation pattern. B-mode US imaging in real time maps the multiple wave dispersion velocities and subsequently theμ is estimated. Although applied in a transverse anisotropic medium, SSI has been mathematically demonstrated to be a reliable tool. Musculoskeletal use for SWE(BRUM *et al.*, 2014; ROYER *et al.*, 2011) is relatively new and to our knowledge the investigation of PT mechanical properties using SSI is incipient with very limited attention addressed to the tendon remodeling process.

2.1.4. Supersonic Shearwave Imaging basic science and evolution

SSI is the result of decades of evolution of studies evaluating stiffness in biological tissues in vivo which started in the 1990s(OPHIR *et al.*, 1991). Called elastography, the ultrasound technique by Strain Imaging (quasi-static) depended on the application of a manual external compression in the region of interest and in the comparison of the images before and after the application of the compression. The more rigid tissues deform less than the less rigid areas and this way the qualitative information of stiffness in the region are obtained(OPHIR *et al.*, 1991).The SI technique is ineffective for the quantification of tissue stiffnessdepending on the differentiation of the acoustic impedance between the evaluated tissues, which is a function of the volumetric modulus of the tissue. Due to the low variability of the volumetric modulus between different biological tissues, it allows only a qualitative character to the technique, limiting its application(EBY *et al.*, 2013; SARVAZYAN *et al.*, 1998).

Due to the inconvenient of the necessity for an unknown and uncontrolled external compressive force, other techniques of ultrasound elastography have been developed and are known as dynamic methods, such as transient elastography, Acoustic Radiation Force Impulse Imaging (ARFI) and Shear Wave Elasticity Imaging (SWE). Transient elastography detects the velocity of the shear waves produced by a low frequency vibration performed by the transducer. Transient elastography is performed in 1-D, that is, it has no image generation purposes, since quantitative information on stiffness is unidimensional(PARKER, 2011; TALJANOVIC *et al.*, 2017)

In ARFI, pulses are generated by the piezoelectric elements in the transducer, creating an acoustic radiation force. The pulses form a bundle in the axial direction that "pushes" the tissue located along the axis of the beam. The less rigid the tissue, the greater the quantity will be displaced, and thus a stiffness map of the analyzed region is constructed by scanning the area of interest with the transducer. In spite of presenting better resolution than the IS technique, the ARFI elastography is only able to provide qualitative information.

In SWE, acoustic radiation generates pulses disturbing the tissue, but it provides information about the stiffness by detecting the speed with which the shear waves generated by the pulse propagate laterally in the tissue. Through the analysis of the velocity of the shear waves, we can estimate the $\mu$of the tissues, which parameter is strongly correlated with the rigidity of the material, allowing its quantification. Due to the great variability of elastic moduli between biological tissues, the distinction and characterization between them becomes more precise(SARVAZYAN *et al.*, 1998).

Finally, SSI uses the same operating principle as SWE, but its ultra-fast data acquisition system allows the characterization of biological tissue in real time(BERCOFF; TANTER; FINK, 2004), which attributes to this technique a special value for analysis of stiffness in vivo.SSI is receiving crescent interest as a tool for musculoskeletal tissues analysis, both in basic research as in clinical settings. One of the main reasons for this evolving interest is the possibility to perform not only a qualitative but also a quantitative analysis. Shear waves creates a movement in adjacent particles transverse to wave propagation. By changing the shape of a substance without changing volume, two equal forces working in opposite directions will disturb the superficial layer. Once it returns to its original shape, adjacent lawyers will undergo shear, propagating the transverse shear wave (SARVAZYAN *et al.*, 1998).

The SSI system operates in two distinct phases: pushing and imaging. In the pushing phase, an acoustic radiation force (ARF) is generated by an electronic transducer at different depths. This force creates shear waves and the constructive interference generate a Mach cone, representing a quasi-planar shear wave propagation (Fig. 10)(BERCOFF; TANTER; FINK, 2004).For a dissipative medium, one has the relation:

$$ARF\ (\vec{r},t) = \frac{2\alpha I\ (\vec{r},t)}{c}$$

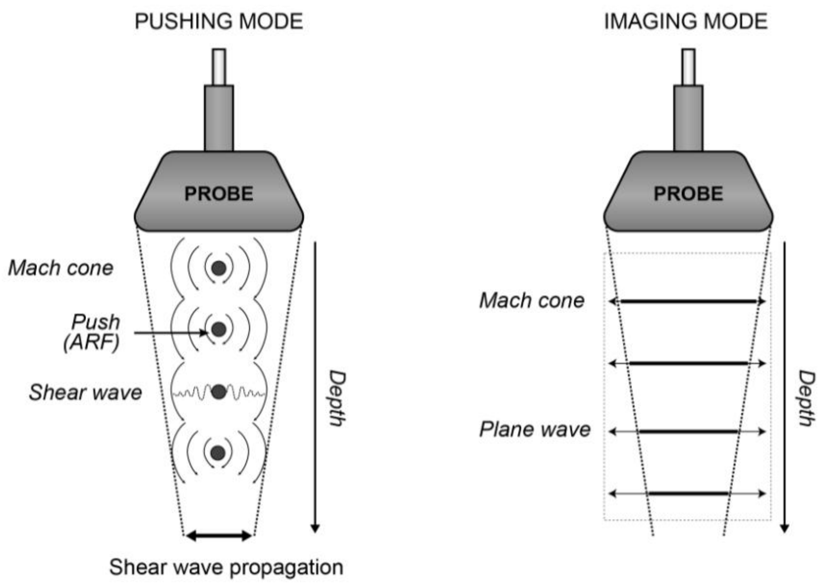

Figure 10. Mach cone generated in pushing fase(adapted from BERCOFF; TANTER; FINK, 2004 and LIMA *et al.*, 2017).

In imaging phase, the transducer captures the vibration of the medium created by the shear wave propagation. Next, an algorithm is applied to all images acquired, determining the degree of displacement in time and the shear wave speed. Finally, the medium μ can be estimated (TALJANOVIC *et al.*, 2017).In a purely elastic and isotropic medium, the c is directly related to the μ in $\mu = pc^2$(COBBOLD, 2007), where p is the biological tissue density, assumed similar to the water (1,000 kg/m^3) (SARVAZYAN *et al.*, 1998). For incompressible media, including most biological tissues, μ is approximately one-third of the E (μ=E/3) (ROYER *et al.*, 2011). Therefore, assuming a purely elastic medium, the shear wave speed can be used to obtain the E according to $E=3\rho c^2$ (ROYER; DIEULESAINT, 2000), which means that the values of the Young's modulus increase as the shear wave speed increases.

SSI assumes the mean to be isotropic, homogeneous and uncompressible. It´s easy to imagine that living tissues that offer physical properties similar to these will represent the ideal mean for analysis. Indeed, SSI has been successfully used determine *in vivo* and non-invasively the mechanical properties of living tissue as the liver (BAVU *et al.*, 2011; PALMERI *et al.*, 2008; SANDRIN *et al.*, 2003). Extending its application to evaluate tendon´s μ can be limited by the understanding on the physics of the shear wave propagation in such complex medium.Tendons may be described as a unidirectional arrangement of collagen fibers within a supporting matrix.

This tridimensional arrangement has none of the settings assumed by SSI. Therefore, the isotropic solid model used in most shear wave elastography techniques is no longer valid and a transverse isotropic model is more suitable (BRUM *et al.*, 2014). This property is assigned to the plane parallel to the orientation of the tendon fibers (Fig. 11) (GENNISSON, J.-L. *et al.*, 2003). Therefore, the proposed approach for determination of tendon stiffness through shear wave analysis remains valid, as demonstrated by previous studies(HELFENSTEIN-DIDIER *et al.*, 2016; LE SANT *et al.*, 2015).

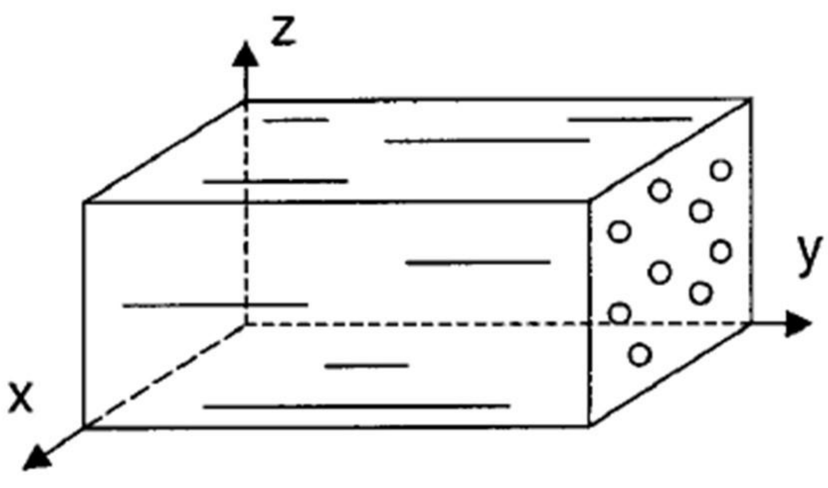

Figure 11. Transverse isotropic modelling of tendon tissue(adapted from GENNISSON, J.-L. *et al.*, 2003).

Lastly, a relevant aspect about SSI has recently received attention. Tendons are characterized by a marked stiffness in the 400 to 1300 kPa range (i.e. fast shear waves). Hence, the shear wavelengths are greater than the tendon thickness leading to guided wave propagation(BRUM *et al.*, 2014) (Fig. 12). These authors suggest that their results demonstrate the importance of a dispersion model analysis in order to properly estimate the tendon elasticity. In another study, using this correction revealed a significantly higher shear moduli C_{55} obtained with the phase velocity mode compared toμ in commercial mode ($p< 0.001$). However, a strong and significant correlation between both values was observed($r= 0.844$, $p< 0.001$) (HELFENSTEIN-DIDIER *et al.*, 2016).

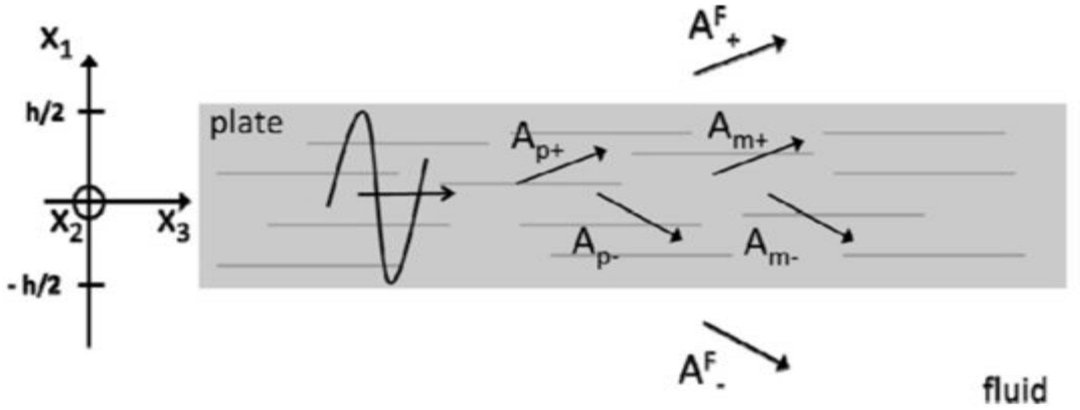

Figure 12. Shear waves propagation parallel to tendon fibers(adapted from BRUM *et al.*, 2014).

2.1.5. Patellar tendon adaptations to resistance training

It´s widely accepted that RT is an important strategy for developing and maintain muscle strength and hypertrophy(AMERICAN COLLEGE OF SPORTS MEDICINE, 2009). Although RT focus is primarily on promoting muscle adaptations, tendons also seems to benefit from overloading. It´s frequently reported changes in cross sectional area (CSA) and stiffness secondary to training (GROSSET *et al.*, 2014; KONGSGAARD *et al.*, 2007; KUBO *et al.*, 2001; MALLIARAS *et al.*, 2013; REEVES; MAGANARIS; NARICI, 2003; SEYNNES *et al.*, 2009).

Kubo et al. (2001) studied this fact for the first time in humans in eight untrained subjects who completed a 12-week (four days/week) isometric training that consisted of unilateral knee extension at 70% of maximal voluntary contraction (MVC) for 20 seconds per set (four sets/day). RT significantly improved MVC torque, the rate of force development and PT E and stiffness. The authors suggest that isometric RT produce changes in tendon mechanical properties making it more efficient for force transmission.

PT anatomy changes seem to depend on RT intensity (KONGSGAARD *et al.*, 2007). In this study, twelve healthy untrained young men were submitted to 12-week RT, three times a week, consisting of 10 sets knee extensions with one leg selected for heavy training (70% 1RM, eight repetitions (REP) three minutes rest interval (RI)) and the other leg to light training (36 REP, 30 seconds RI, load selected to equal the amount of work performed by the heavy RT leg). Only the knee extensors submitted to heavy RT exhibited significant increases in MVC and PT stiffness. The PT CSA increased significantly in proximal and distal section in the heavy RT legs and only in the proximal third in the light RT leg.

Malliaras et al. (2013) also studied the effect of different intensities and contraction types in untrained healthy young men. B mode US and dynamometry were used to measure PT stiffness and E pre and post training. One group was assigned as control and three groups were tasked for a 12-week, three time/week knee extension RT consisting of: 1- concentric (80% of concentric–eccentric 1RM, 4 sets, 7–8 REP), 2- standard load eccentric only (80% of concentric-eccentric 1RM, 4 sets, 12–15 REP) and 3- high load eccentric (80% of eccentric 1RM, 4 sets, 7–8 REP). All groups increased PT stiffness and E after training compared to the control group. No significant differences were observed between groups, although the high load eccentric group increase in PT E was greater than in the low load eccentric group (84-87% x 59%). The authors argue that the small sample size might be a study limitation that would impair finding significant results (nine to 10 subjects in each group).

These RT effects have also been demonstrated in the elderly. Analyzing this fact for the first time in this specific population, Reeves et al. (2003) compared two groups of untrained old men and women, one submitted to RT and the other assigned as a control group. The RT protocol consisted of two sets of leg press and knee extensions performed three times a week for 14 weeks at 80% of 5RM and a three-minute RI. Training increased tendon stiffness 65%, E 69% and torque development 27% measured with B mode US evaluation in isometric contractions. No changes were registered in the control group.

Later, Grosset et al. (2014) studied the effects of RT intensity in the elderly. A group of 20 old men and women (17 completed the RT protocol) were randomly assigned to engage in a 12-week RT protocol at low (40% 1RM) or high intensity (80% 1RM) three days/week. Two to four sets were performed with a three-minute RI. Only the high intensity group exhibited increase in tendon stiffness and E suggesting that for a mixed gender elderly population, low intensity exercise prescription may not be sufficient to affect tendon properties.

Although it seems consent that RT improves PT stiffness and quadriceps strength, one particular study has questioned if the changes are more closely related to gains in strength or to muscle hypertrophy (SEYNNES *et al.*, 2009). In this study, 15 untrained healthy young men were submitted to a nine-week RT protocol consisting of knee extensions (four sets at 80% 1RM, 10 REP, two minutes RI). Quadriceps physiological cross-sectional area (PCSA) and MVC isometric strength increased 31% and 7% respectively. Tendon CSA and tendon stiffness and modulus increased 24% and 20% respectively. A moderate positive correlation was observed between muscle PCSA and tendon stiffness (r= 0.68) and E (r= 0.75) and none of these adaptations were related to strength gains. Unexpectedly, the increase in muscle PCSA was inversely related to the distal and the mean increases in tendon CSA (in both cases, r= -0.64). The authors argue that these data suggest that following short-term RT, changes in tendon mechanical and material properties are more closely related to the overall loading history and that tendon hypertrophy is driven by other mechanisms than those eliciting tendon stiffening.

2.1.6. Patellar tendon adaptations to specific loading environments

If RT seems to improve PT mechanics, stress shielding was associated with reduction in tendon stiffness (COUPPÉ *et al.*, 2012; RUMIAN *et al.*, 2009). Rumian et al. (2009) first showed this effect in 12 sheep using an external fixator device unilaterally to bypass the quadriceps force that would be usually transferred to PT. After six weeks of stress shielding, a significant reduction in the PT stiffness (79%), E (76%), ultimate load (69%), energy absorbed (61%) and ultimate stress (72%), was observed when comparing to the contra-lateral side. The authors notice that this reflects the important relationship between stress and mechanical properties in tendons, but that these findings may not be perfectly suited for humans.

Trying to answer this question Couppé et al. (2012) studied eight young and eight elderly men going on 14 days of unilateral leg immobilization. All individuals´ PT were assessed bilaterally before and after intervention. In the older individual's immobilization decreased tendon stiffness and E in both sides, while in young individual's tendon stiffness and E decreased only in the immobilized leg.

It´s worth noting that muscle adaptations happens quicker than tendons´ and therefore chronic studies trying to correlate gains in muscle strength and changes in PT mechanical properties after training can be limited by the nature of different structures remodeling rate (WIESINGER *et al.*, 2015). In that context, habitual loading environments were studied and have also shown to promote adaptations in the PT properties as well (COUPPE *et al.*, 2008; KUBO *et al.*, 2010; SEYNNES *et al.*, 2013; WESTH *et al.*, 2008; ZHANG, Z. J.; NG; FU, 2015).

Couppé et al. (2008) analyzed this particular effect in athletes submitted to different habitual loading between legs. A group of seven elite fencers and badminton players with side-to-side strength difference greater than 15% was evaluated in a cross-sectional study. The stronger leg revealed a stiffer and thicker PT without differences in E. This data showed that a habitual loading is associated with increased PT size and mechanical properties. The authors suggest that higher loading promote chronic adaptations in tendon size and mechanical properties E changes.

The positive correlation between KT and PT stiffness and E has also been reported in subjects chronically adapted to heavy RT (SEYNNES *et al.*, 2013). When comparing three groups, 1- RT subjects, 2- RT subjects using androgenic-anabolic steroids and 3- control, the authors found significant differences in isometric torque, tendon stiffness and elastic modulus. The higher values for PT mechanical properties were found in the RT using androgenic-anabolic steroids group.

Although strength and KT are important variables influencing PT mechanics, the loading pattern also seems to plays a determinant role in structural adaptations. Examining long distance male runners (5000 meters), Kubo et al. (2010) detected a significant difference in MVC relative to body weight favoring the control group composed of untrained men. Although stronger, the control group exhibited lower PT stiffness. Moreover, when examining long distance records and PT stiffness in the long-distance runners, a significant positive correlation was observed. The authors hypothesize that a stiffer PT was more efficient in transmitting muscle forces determining greater running performance (KUBO *et al.*, 2010).

Contrasting findings however, were reported in volleyball and basketball players compared with a sedentary group (ZHANG, Z. J.; NG; FU, 2015). In these high impact sports, the PT exhibited lower stiffness and E, but higher CSA and thickness. These different established biological responses to different chronic mechanical stimulus indicate adaptations reflect the kind of loading received. Although questionable in some high impact environments, it seems that a stronger knee extensor mechanism is correlated to a stiffer and less elastic PT.

2.2. Hypothesis

Based on previous literature showing an increase in PT E after resistance training protocols(GROSSET et al., 2014, KUBO et al., 2001, MALLIARAS et al., 2013) and based on thedirect relation between E and the Lamé´s constants (μ and λ), our initial hypothesis is that RT will increase PT E and this will be reflected asincreased μ values in SSI evaluation.

We also hypothesize that the RT directed to the knee extensor mechanism and quadriceps will increase KT and VL MT(AMERICAN COLLEGE OF SPORTS MEDICINE, 2009). Due to the short term intervention (< 6 months) however, no changes in PT morphology are expected(WIESINGER *et al.*, 2015). Lastly, based on previous results seem in chronic RT interventions on the triceps brachii and biceps femoris (AKAGI *et al.*, 2016; SEYMORE *et al.*, 2017), we don´t expect significant changes in the VL μ to be detected by SSI.

2.3. Objectives

2.3.1. Main Objective

The main objective of our present study is to evaluate the PT mechanical properties adaptations, expressed by μ changes in SSI evaluation, before and after a resistance training protocolin untrained healthy young men.

2.3.2. Secondary Objectives

Some secondary objectives were identified:

1- to measure the effectiveness of the RT protocol, by assessing quadriceps hypertrophy and strength expressed by VL MT and KT, before and after the 8-week intervention.

2- to evaluate changes in PT morphology secondary to RT expressed by PTT, as a way to scan the repercussion of the guided waves effect in PT µ evaluation.

3- to analyze the VL µ before and after the RT protocol, observing the extensor mechanism mechanical response as a unit to controlled chronic overloading.

4- to examine the reliability of SSI evaluation,calculating the intra-class correlation coefficients of the PT µ and VL µ in the images obtained.

5- to analyze if PT µ oscillations are correlated to changes in the correspondent muscle function (KT), hypertrophy (VL MT) or mechanical properties (VL µ).

3. MATERIALS AND METHODS

3.1. Ethics statement

The University Hospital Ethics Committee approved this study (registration number 2.811.595). The experimental procedures were conducted in accordance with the Declaration of Helsinki and to the resolutions 196/96, 466/12 and 510/16 in compliance to the Plataforma Brasil. All participants received instructions about the study procedures and provided informed written consent before testing.

3.2. Experimental procedure

The study was conducted at the Biomedical Engineering Department of the Federal University of Rio de Janeiro (UFRJ) between july 2017 and august 2018. The body weight and height of all subjects were measured and body mass index (BMI) was calculated. Age was registered and the dominant leg wasself-informed by participants. All subjects' PT and VL were submitted to SSI evaluation pre and post intervention. As a form to assure that the RT protocol was effective, Vastus Lateralis muscle thickness (VL MT) and knee extensor torque (KT) were measured at baseline and after the eight weeks. PT thickness (PTT) was measured pre and post intervention to detect possible tendon structural adaptations. All post intervention measures were performed one week after the last training session.

The intervention and data acquisition protocol are available in dx.doi.org/10.17504/protocols.io.ykwfuxe.

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3.3. Subjects

In this longitudinal study, untrained male volunteers had the right knee examined (table 1). Age was set between 25 and 40 years old to eliminate any variation of PT properties due to age or gender(HSIAO *et al.*, 2015; ONAMBELE; BURGESS; PEARSON, 2007). Once no previous studies using SSI to evaluate tendons µ responses to RT were found, it was not possible to estimate a necessary number of subjects calculated by an acceptable beta error. Therefore, the number of 15 subjects was selected based on previous literature evaluating the PT mechanical response to RT with B-mode US combined with dynamometry (WIESINGER *et al.*, 2015). None of the subjects had participated in any systematic training or physical activity for at least 6 months. Any clinical history or report of knee pain/injuries, systemic disease or previous knee surgery was considered as exclusion criteria. All subjects were right handed.

Table 1. Demographic characteristics.

Variables	n = 15
Age (years)	28.67± 3.26
Height (cm)	177.33± 6.88
Weight (kg)	91.83± 17.25
BMI (kg/m^2)	28.84 ± 4.44

Values shown as mean ± SD. BMI = body mass index.

3.4. Resistance training protocol

Participants were designated to 8-week resistance training for the quadriceps femoris muscle consisting of free-weight Squats (SQ) and Knee Extensions (KE) in a knee extension machine (MatFitness®, São Paulo, Brazil) in this precise exercise order. RT protocol was designed based on the ACSM recommendations for healthy individuals and adapted based on previous studies with similar design (AMERICAN COLLEGE OF SPORTS MEDICINE, 2009; BOHM; MERSMANN; ARAMPATZIS, 2015). The frequency of the training program was 2 sessions per week with at least 72 hours rest interval between sessions. A total of 16 sessions were performed in the 8-week training period with all the sessions occurring between 8 and 10 AM.

At baseline, 10RM testing was performed for both exercises. All subjects were submitted to a familiarization before testing during which the subjects performed the same exercises as used in the 10RM tests with the aim of standardizing the technique of each exercise. The 10 RM tests and retest were then performed on 2 nonconsecutive days separated by 48-72 hours. The heaviest resistance load achieved on either of the test days was considered the pre-training 10RM of a given exercise. The 10RM was determined in no more than five attempts, with a RI of five minutes between attempts and a 10-minute recovery period was allowed before the start of the testing of the next exercise(SIMÃO *et al.*, 2007).

The 10RM tests were used to set the initial training load. Subjects were instructed to perform both exercises to complete concentric failure (inability to perform muscle shortening against external resistance) in all sets. The weighs were continually adjusted to keep the exercises in an 8-12 repetitions range, with a two-minute RI between sets. Full range of motion was used in both SQ and KE. The RT program followed a linear periodization with progressive volume. Four sets were performed per exercise in weeks 1-4 and six sets per exercise in weeks 5-8 (table 2). Before each training session, the participants performed a specific warm-up, consisting of 20 repetitions at approximately 50% of the resistance used in the first exercise of the training session (SQ). Contraction time was self-determined as individuals were instructed to perform both exercises until concentric failure in the 8-12 repetition range with the highest load possible. Adherence to the program was superior to 90% in all individuals and a strength and conditioning professional and a physician supervised all the training sessions. Verbal encouragement was provided during all training sessions.

Table 2. Resistance Training Protocol.

Week	Session/Week	Set X Repetition
Familiarization	2	
10RM test and retest	2	10RM test and retest
1-4	2	4 X 8-12
5-8	2	6 X 8-12

3.5. Measurement of patellar tendon shear modulus and thickness

An Aixplorer US (v.11, Supersonic Imaging, Aix-en-Provence, France) with a 60-mm linear-array transducer at 4–15 MHz frequency was used in this study. The transducer was positioned at the inferior pole of patella and aligned with the patellar tendon, with no pressure on top of a generous amount of coupling gel. B-mode was used to locate and align the PT longitudinally. When a clear image of the PT was captured, the shear wave elastography mode was then activated. The transducer was kept stationary for approximately 10 seconds during the acquisition of the SWE map. A total of four images were acquired and saved for off-line processing analysis. Scanning of PT was performed with the subject in supine lying and the knee at 30° of flexion (ZHANG, ZHI JIE; FU, 2013). The knee was supported on a custom-made knee stabilizer to keep the leg in neutral alignment on the coronal and transverse planes (Fig. 13). Prior to testing, the subjects were allowed to have a 10-min rest to ensure the mechanical properties of PT were evaluated at resting status. The room temperature was controlled at 20° C for all image acquisitions.

Figure 13. Imaging acquisition with the knee resting over a custom-made support at 30^{o}(KOT *et al.*, 2012).

The Q-box selected was the larger possible rectangle in order to consider more PT elasticity information. The µ values were obtained by a custom MatLab® routine and ROI limits were defined as the area between 5 and 25 mm from the inferior pole of the patella excluding the paratendon (Fig.14) (MANNARINO, P. *et al.*, 2017). The custom routine calculated the µ by dividing the mean E generated from the system by 3 (ROYER *et al.*, 2011).

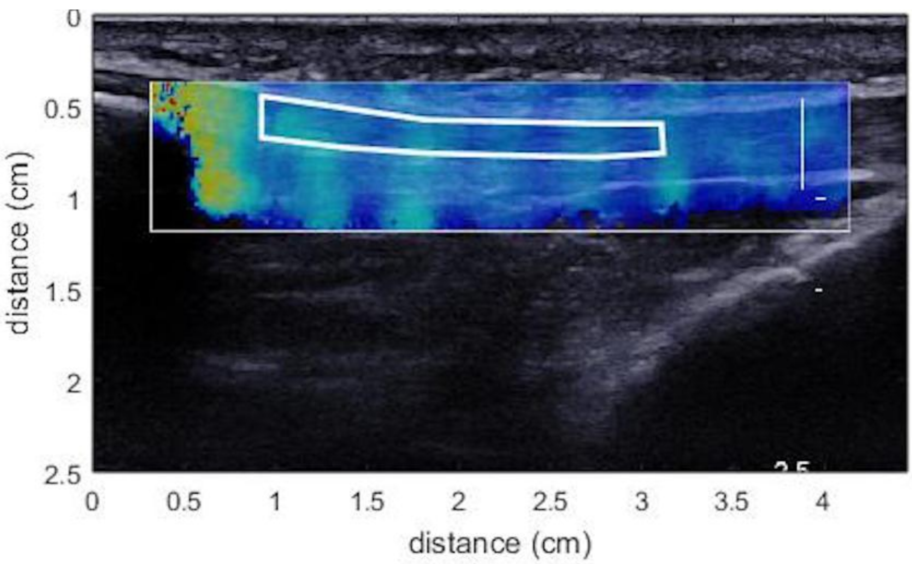

Figure 14.MatLab® custom routine and ROI defined between 5 and 25 mm from the patella tip(MANNARINO, P. *et al.*, 2017).

Off-line analysis using ImageJ® 1.43u (National Institutes of Health, Bethesda, MD, USA) was performed with using two B-mode recorded images and the mean values were considered for analysis. PTT was measured at 20 mm from the inferior pole of the patella. The measure was limited by the PT deep and superficial paratendon and oriented transversely to the tendon fibers (Fig. 15).

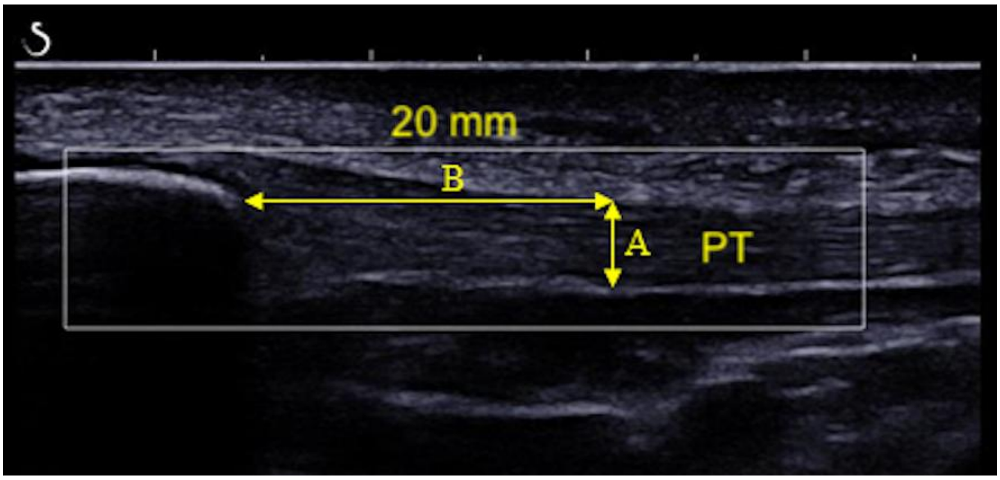

Figure 15. ImageJ® measure of PTT at 20 mm from the inferior pole of the patella(MANNARINO, PIETRO; MATTA; OLIVEIRA, 2019).

3.6. Measurement of vastus lateralis shear modulus

The same equipment was used for VL μ measurement. A longitudinal line was drawn between the most superficial and palpable portion of the great trochanter and the lateral epicondyle. Scans were taken at 50% of the length of the line (BLAZEVICH *et al.*, 2009). The line length and distance from the great trochanter where the imaging was performed was registered for every volunteer to ensure that the post intervention analysis was made in the same exact location in the. B-mode was used to locate and align the probe with the VL. The images were recorded with subjects lying supine with their knee fully extended and their muscles fully relaxed. When a clear image of the VL was captured, the SWE mode was then activated. A total of four images were acquired and saved for off-line processing analysis.The ROI was selected avoiding any detectable vascular structure within the muscle and the deep fascia and based on the quality map (Fig.16).

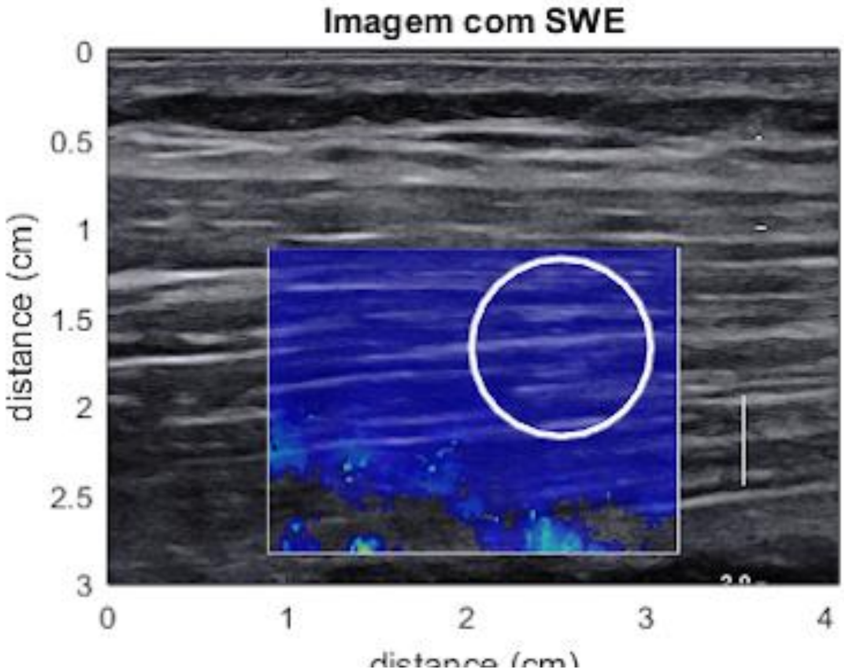

Figure 16. VL µmeasurement and selected ROI.

3.7. Measurement of vastus lateralis muscle thickness

The images acquisition was performed using a US (GE LogiqE, Healthcare, EUA), frequency of10 MHz, for longitudinal scans of the VL muscle. The US probe was centered and the images were recorded with subjects on the same position and location used for VL SWE. The VL images were obtained on longitudinal plane laterally and the MT was determined as the mean of three distances (proximal, middle and distal) between superficial and deep aponeurosis for each image (MATTA *et al.*, 2017) (Fig.17). The images were processed with publicly available software (ImageJ 1.43u; National Institutes of Health, Bethesda, MD, USA). For each image, two consecutive measurements were performed and the mean values were considered for analysis.

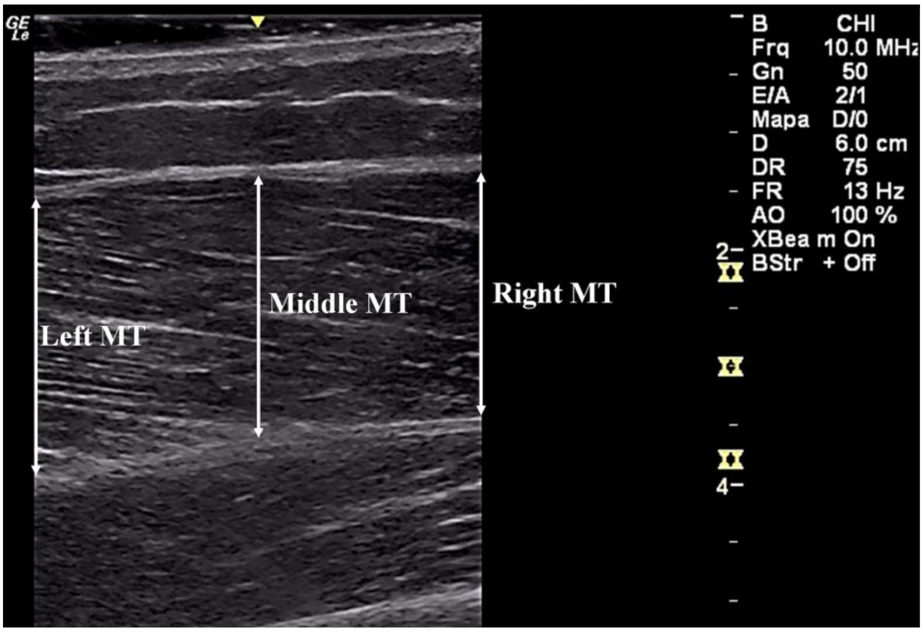

Figure17. VL MT measurement with B-mode US.

3.8. Measurement of knee extension torque

The maximal isometric extension KT was measured with an isokinetic dynamometer (BIODEX®, Biodex Medical Systems, Shirley, NY, USA) at 80° of knee flexion (BLAZEVICH *et al.*, 2009). Subjects were positioned seated with inextensible straps fastened around the waist, trunk and distal part of the thigh. The backrest inclination and seat translation as well as the dynamometer height were adjusted for each subject, to ensure proper alignment of the rotation axis of the dynamometer with the lateral condyle of the femur (Fig. 18). The right knee was fixed to the dynamometer lever arm 5 cm above the lateral malleolus. Settings were recorded for re-test reproducibility. After a specific warm-up consisting of two submaximal isometric knee extensions, the subjects performed two 5-s maximal voluntary isometric contractions (MVIC) with one-minute rest between trials. Subjects were verbally encouraged to reach maximal effort while a visual feedback of the torque level was provided. The highest peak torque among trials (corrected for gravity) was recorded for analysis.

Figure 18. Subject positioning for MVIC examination in the BIODEX®.

3.9. Statistical Analysis

Descriptive data such as mean ± standard deviation (SD) were calculated. The software GraphPad Prism 7® (Graphpad software Inc., La Jolla, CA, USA) was used for statistical analysis. After the normality distributions were verified using the Shapiro-Wilk tests, paired t-tests were used to compare the PT µ, PTT, VL µ, VL MT and KT at baseline and after the RT protocol.Pearson's correlation coefficient was used to investigate the association between the PT µ changes and PTT, VL µ, VL MT and KT oscillations ((POST - PRE)/PRE). SSI reliability of PT µ and VL µwere examined using intra-class correlation coefficients (ICC).Based on the 95% confident interval (CI) of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively (KOO; LI, 2016).A value of $p < 0.05$ was adopted as statistically significant.

4. RESULTS

4.1.Shear modulus intra-class coefficient

ICC values for PT µ and VL µ were calculated and reliability was rated good and excellent, respectively (table 3).

Table 3. Intra-class coefficient values.

	ICC	95% CI		p-value
PT µ	0.877	.647	.990	<.000
VL µ	0.962	.865	.990	<.000

4.2.Patellar tendon and vastus lateralis shear modulus

No statistically significant changes in PT µ were observed after the eight weeks of RT (baseline= 78.85 ± 7.37 kPa and post = 66.41 ± 7.25 kPa, p = 0.1287) (Fig. 19).

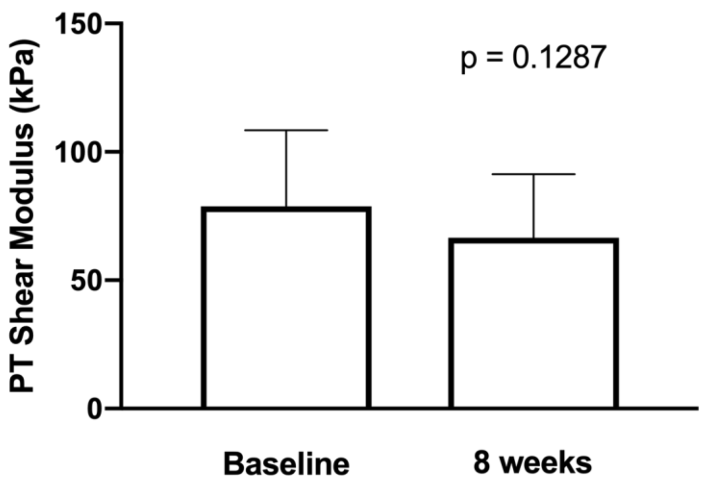

Figure 19. 7 PT µ at baseline and after eight weeks of resistance training.

A statistically significant increase in VL was observed after the eight weeks of RT (baseline = 4.87 ± 1.38 kPa and post = 9.08.12 ± 1.86 kPa, p = 0.0105) (Fig. 20).

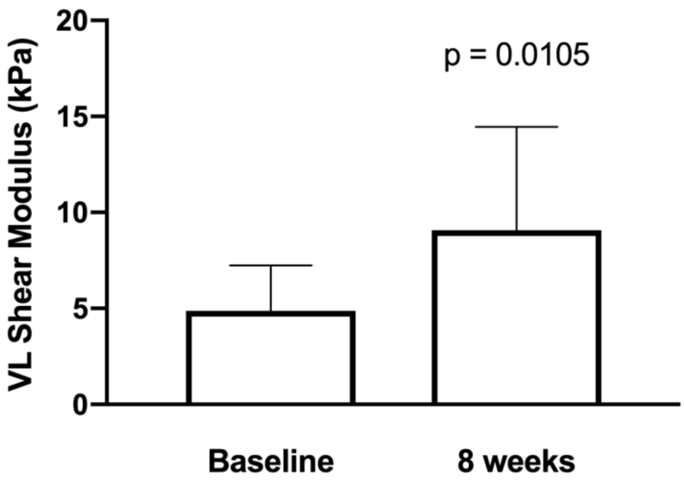

Figure 20.VL μ at baseline and after eight weeks of resistance training.

4.3. Patellar tendon and vastus lateralis thickness

A statistically significant increase was observed in VL MT after the resistance training protocol (baseline = 2.40 ± 0.40 cm and post = 2.63 ± 0.35 cm, p = 0.0112) (Fig. 21).

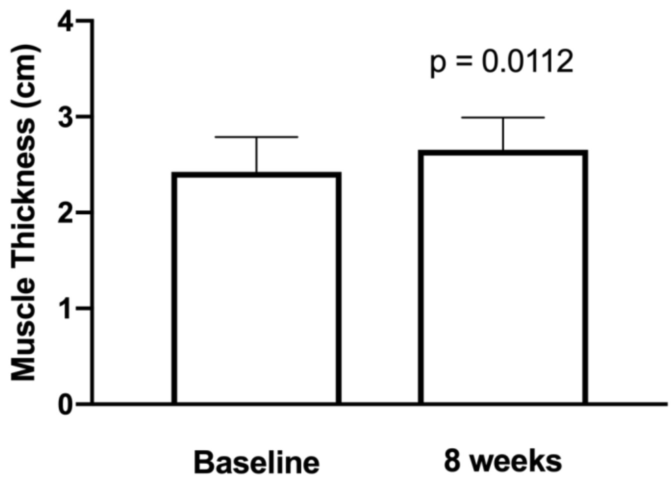

Figure 21. VL MT at baseline and after eight weeks of resistance training.

No statistically significant changes in PTT were observed after the eight weeks of RT (baseline= 0.364 ± 0.053 cm and post = 0.368 ± 0.046 cm, p = 0.71) (Fig. 22).

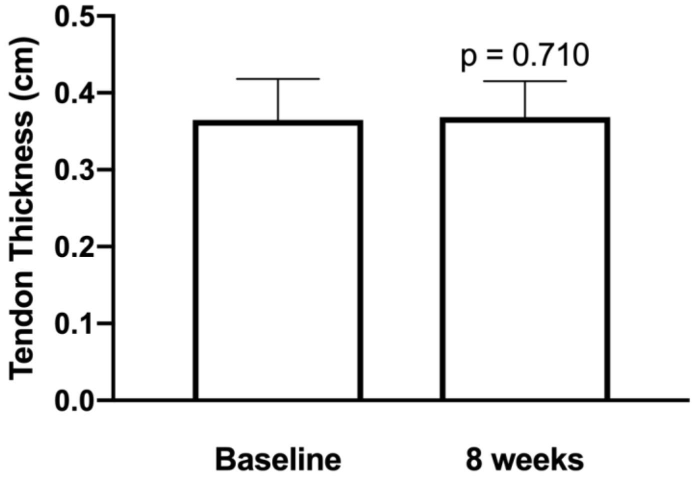

Figure 22. PTT at baseline and after eight weeks of resistance training.

4.4.Knee extensor torque

A statistically significant increase was observed in KT after the resistance training protocol (baseline = 294.66 ± 73.98 Nm and post = 338.93 ± 76.39 Nm, p = 0.005) (Fig. 23).

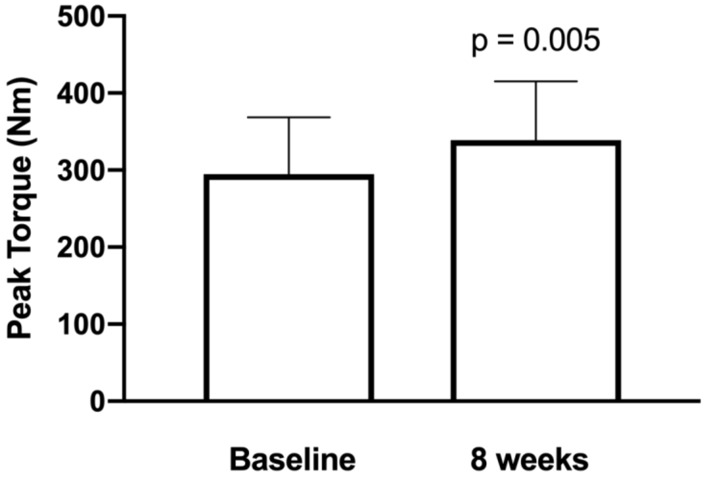

Figure 23.KT at baseline and after eight weeks of resistance training.

4.5. Correlation to patellar tendon modulus changes

No statistically significant correlations were observed between the changes in PTµ and VL µ (table 4).

Table 4. Correlation to patellar tendon modulus changes.

PT μvs.	VL μ	VL MT	KT	PTT
r	-0.136	0.234	0.009	0.419
p-value	0.629	0.441	0.974	0.136

5. DISCUSSION

Our study aimed to assess the effects of a progressive RT protocol directed for the knee extensor mechanism on the PT μ measured by SWE. Our findings indicate that the proposed intervention was effective in promoting quadriceps hypertrophy and strength gains, as VL MT and KT increased significantly (p=0.0112 and p= 0.005, respectively). Also, the intervention was effective in promoting VL μ adaptations (p= 0.0105), but not in significantly affecting PT μ (p= 0.1287) nor PTT (p= 0.71), which can reflect a faster response to RT from muscle than from tendons. No statistically significant correlations were observed between the changes in PTμ and VL μ (r= -0.136, p= 0.629), VL MT (r= 0.234, p= 0.441), KT (r= 0.009, p= 0.974) or PTT (r= 0.419, p= 0.136).

The study of the tendons and muscle adaptation process to progressive overload is fundamental to design optimal strategies directed to injury prevention and rehabilitation (THOMOPOULOS *et al.*, 2015). Changes it tendon composition, architecture and collagen cross-linking determined by resistance training can impact ultimate force to failure and resistance to injury (MARTIN *et al.*, 2015; YEH *et al.*, 2016).These adaptations can be reflected in variations of tendon stiffness, E andμ.

Two recent literature reviews investigated the PT mechanical properties changes after short-term (6-14 weeks) RT protocols (BOHM; MERSMANN; ARAMPATZIS, 2015; WIESINGER *et al.*, 2015). Significant increase in PT stiffness and E were observed routinely as a consequence of RT (GROSSET *et al.*, 2014; KONGSGAARD *et al.*, 2007; MALLIARAS *et al.*, 2013; SEYNNES *et al.*, 2009). It is important to notice that these previous literature addressing the changes in PT mechanical properties secondary to RT were based exclusively on stress and strain estimation derived from B-mode US measures and dynamometry during isometric quadriceps contraction (WIESINGER *et al.*, 2015).

Although extensively documented, there is no consensus related to the acquisition protocol and this methodology can jeopardize results previously found (BURGESS *et al.*, 2009; PEARSON; BURGESS; ONAMBELE, 2007; SEYNNES *et al.*, 2015). Many technical details can limit these results reliability such as limited scanning from narrow fields of view (HANSEN *et al.*, 2006; MAGANARIS, 2005), desynchronization between force production and elongation registration (FINNI *et al.*, 2012), limited three dimensional tracking of anatomical landmarks during muscle contraction (IWANUMA *et al.*, 2011; MAGANARIS, 2005; PETER MAGNUSSON *et al.*, 2001), tendon force estimation inaccuracies (SEYNNES *et al.*, 2013) and others.

Due to these practical limitations, we used SWE to observe the changes PT mechanical properties expressed by PT μ, before and after a RT protocol. To our knowledge, this is the first work directed to PT with this design. For research purpose, muscle-tendon units are characterized as transverse isotropic, due to the its fiber alignment (GENNISSON, J.-L. *et al.*, 2003). Nevertheless, SWE was externally validated and PT μ showed a strong positive correlation to longitudinal Young´s Modulus (E), ultimate force to failure and resistance to tensile loading in *in vitro* models (MARTIN *et al.*, 2015; YEH *et al.*, 2016), so we expected an increase in PT μ after the intervention.

Despite previous literature consistently demonstrating that RT interventions increased PT E and stiffness(WIESINGER *et al.*, 2015), the present study revealed no changes in PT μ after the 8-week RT protocol (p= 0.1287). Assuming the PT as a hexagonal system and considering that in soft media $\lambda$is 10^6 greater than μ, it was expected a direct linear relation between E and μ ($E \cong 3\mu$) (GENNISSON, J.-L. *et al.*, 2003), which was not observed. As the RT intervention could be considered effective, as reflected by quadriceps hypertrophy and strength gains (KT, p=0.005 and VL MT, p= 0.0112), some possible explanations for this result can be hypothesized.

First, analyzing our protocol duration, the 8-week intervention could be not sufficient to trigger changes in tendon mechanical properties at an extent to make the μ detectable by SWE. It has been previously reported that tendon remodeling process secondary to RT protocols can take longer periods (WIESINGER *et al.*, 2015), comparing to muscle adaptations. Although literature on the topic uses RT protocols lasting 6-14 weeks, a longer intervention could be necessary to make μ subtle changes detectable by SWE.

Second, the changes in resting state passive tension in the muscle-tendon unit deserves particular attention. It was previously reported that the μ presents strong correlation to the tangent traction modulus at the time of SWE image acquisition (FONTENELLE *et al.*, 2018; ZHANG, ZHI JIE; FU, 2013). It is also documented that RT can increase flexibility (MORTON *et al.*, 2011) and that static stretching was able to reduce de E and μ acutely (HIRATA *et al.*, 2016; PAMBORIS *et al.*, 2018). This could mask the resistance training effects on PT μ. In one hand the expected increased collagen synthesis and tendon stiffening would increaseμ, while in the other hand the relaxation in the muscle-tendon unit and reduction in passive tension applied to the tendon could reduce it.

Lastly, our sample size and characteristics (15 individuals) was compatible to previous studies previously published investigating PT adaptations to RT protocols with B-mode USand dynamometry (KONGSGAARD *et al.*, 2007; KUBO *et al.*, 2001). However, the known wide range of normal PT µ values (KOT *et al.*, 2012; MANNARINO, P. *et al.*, 2017; ZHANG, Z. J.; NG; FU, 2015)implies a larger SD in PT µ values registeredpre and post intervention. This may require a larger sample to be tested in future studies trying to reach statistically significant results.

Due to this wide range of normal PT µ, central tendency measures could mask individual responses to the proposed intervention. As a way to analyze if there were responders´and non-responders´ subjects that could present any association between the PT µ changes after the RT protocol and the others variables registered (VL MT, VL µ, KT and PTT), the correlation between each ofthese data was tested individually. No statistically significant correlations were found.

Animportant aspect that should be considered when using SWE for PT mechanical properties estimation is the guided waves effect, which is directly affected by the tendon thickness (HELFENSTEIN-DIDIER *et al.*, 2016). Therefore, changes in PT structure and thickness after RT could represent a bias in PT µ changes analysis pre and post intervention. In our study however, PTT analysis revealed no significant changes after the 8-week RT. This results are in accordance with previous literature that revealed that larger tendons were observed only after longer interventions (months, years) (COUPPE *et al.*, 2008; SEYNNES *et al.*, 2013; WIESINGER *et al.*, 2015). With the unaltered PTT pre and post intervention showed in our study, we can assume that the guiding effect should have impacted similarly the PT µ at baseline and after the RT protocol.

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Muscle mechanical properties seems to be much less explored, mainly by its limiting technical settings (LEVINSON; SHINAGAWA; SATO, 1995; MURAYAMA *et al.*, 2000). Using US plus dynamometry technique does not seems feasible due to the complex architecture when studying pennate muscles or due to the absence of reference anatomical landmarks in fusiform muscles to calculate strain(LIEBER *et al.*, 2017; YANAGISAWA, O. *et al.*, 2011). Until the advent of SWE, muscle mechanical properties were obtained by external mechanical analysis, as the muscle hardness index using a durometer,which is very limited and whose reproducibility has not been systematically evaluated (NIITSU *et al.*, 2011).

In the present study, an important increase in VL μ was observed (p= 0.0105). Some possible explanations for these results can be found. First, the increased VL μ could be resulted from increased collagen content, collagen linking and tissue fluid increasing seem after RT chronic interventions (KOVANEN; SUOMINEN; HEIKKINEN, 1984). Second, although data acquisition was made one week after the last training session, due to the high volume and intensity nature of the RT protocol, it is possible that some residual muscle damage could have impacted the VL μ, increasing its values (YANAGISAWA, OSAMU *et al.*, 2015). Lastly, changes in muscle architecture and pennation angle of the VL can have some impact in the μ, although the magnitude of these changes in the SSI technique is still under investigation (GENNISSON, JEAN LUC *et al.*, 2010a; LIMA *et al.*, 2017). These results, however, contrast with previous findings related by the literature (AKAGI *et al.*, 2016; SEYMORE *et al.*, 2017).

In the literature, SWE values show a strong positive correlation with the muscle force production and activation for quadriceps, triceps surae, abductor minimum and others (ATEŞ *et al.*, 2015; BOUILLARD, K. *et al.*, 2012; BOUILLARD, KILLIAN; NORDEZ; HUG, 2011; KOO *et al.*, 2014), showing that as muscle contract level rises, the more stiffer it becomes. However, the changes in muscle μ secondary to RT are far less studied. To our knowledge, only two studies addressed this topic.

Akagi et al. (2016) reported no changes in the triceps brachii μ after a 6-week RT consisting of triceps extensions (AKAGI *et al.*, 2016). Different from our study however, the authors report that transductor was positioned transversely to muscle fibers, which can actually exhibit lower μ values and blunt differences (EBY, SARAH F., SONG, PENGFEI, CHEN, SHIGAO, CHEN, QINGSHAN, GREENLEAF, JAMES F., AN, 2014; GENNISSON, JEAN LUC *et al.*, 2010b). Furthermore, the study used a shorter intervention (6 weeks) consisting of only one exercise in lower training volume (5 sets), what could imply in smaller adaptations and explain the undetectable changes.

Another study investigated the effects of a 6-week protocol consisting exclusively of eccentric RT (Nordic Curl) on biceps femoris (SEYMORE *et al.*, 2017). Similarly, to Akagi et al. (2016) no statistically significant differences in biceps femoris μ were observed. However, in this study, stretching was also used in the protocol. It was already evidenced that stretching can reduce muscle μ (AKAGI; TAKAHASHI, 2014), so increases in muscle µ secondary to the RT may have been counteracted by the addition of a stretching intervention. Another relevant aspect is that this study involves a fusiform muscle, which architecture can influencedifferently the µ evaluation and is significantly different from the pennate VL(GENNISSON, JEAN LUC *et al.*, 2010a).

Although SWE represents the state of the art in soft tissue mechanical properties evaluation (BERCOFF; TANTER; FINK, 2004; GENNISSON, J. L. *et al.*, 2013), inferring structural adaptations in the musculoskeletal tissues after RT protocols is not trivial. Two different structures with similar composition can present different µ values on SWE evaluation if subjected to different tension during exam. The same applies to structures with different structural arrangement, that can present equal µ values on evaluation if subjected to different tension at the moment of testing (DUBOIS *et al.*, 2015; FONTENELLE *et al.*, 2018; GENNISSON, JEAN LUC *et al.*, 2010b; KOT *et al.*, 2012). We controlled the testing position as much as possible to avoid this influence, trying to guarantee that the muscle was fully relaxed, but it is not possible to guarantee that the relaxed muscle tension pre and post intervention were the same.Further studies researching muscle and tendon mechanical adaptations to RT with SWE should address this question and try to quantify the passive tension in the muscle-tendon unit in the resting state pre and post intervention.

Another relevant limitation of the study is the underestimation of the tendon µ by commercially available SWE equipment previously documented by Helfestein-Didier et al. (HELFENSTEIN-DIDIER *et al.*, 2016). Although the authors report high correlation (r=0.84) between the conventional method and the “corrected” method quantifying shear wave velocities and dispersion analysis, the guided waves generated within the tendon due to its limited thickness can determine statistically significant underestimated µ values. Furthermore, the SWE assumes a transverse isotropic medium, which does not properly represent the complex tendon architecture (BRUM *et al.*, 2014). In our study, no corrections through dispersion analysis were implemented to the SSI evaluation. When studying long term effects, the impact of these combined limitations to results is unknow. Further research in the field should attempt to address this bias.

One point that also deserve attention, is the tendon anatomy and morphology changes in response to the RT. We used the PTT as a way to address tendon structural adaptations, but mostly as a way to guarantee that the guided waves effect that can bias the SWE estimation was not impacting differently pre and post measures(HELFENSTEIN-DIDIER *et al.*, 2016). PTT consists in a unidimensional analysis of the PT structure, what can be considered very limited for this purpose. Also, PTT depends on the examined site, what can be easy to reproduce on the transverse plane based on the distance to the patella tip, but much harder to repeatprecisely on the sagittal plane. Further studies addressing this topic should include not only the PTT pre and post intervention, but also the evaluation of the PT CSA(WIESINGER *et al.*, 2015).

Lastly, the VL pennation angle was not determined pre and post RT in this study. The Aixplorer SSI offers a relatively restricted field of view when compared to conventional US. In larger subjects (present group average= 91.8 ± 17.25 kg), many times it is not possible to visualize the VL deep fascia necessary to calculate the pennation angle. Previous literature has provided evidence that particular attention should be paid to interpreting the SWE data related to the pennate muscles (GENNISSON, JEAN LUC *et al.*, 2010a) as it seems that the fascicle orientation can influence SWE measurements (MIYAMOTO *et al.*, 2015). Also, it is well documented that RT protocols can determine changes in the pennation angle (KAWAKAMI *et al.*, 1995), so it is possible that SWE measurements in chronic studies addressing pennate muscle can suffer influence of the changes in muscle architecture promoted by RT. Further investigation using SWE to address pennate muscles μ changes after RT, should give particular attention to the impact of fascicle orientation changes on SWE measurements.

To our knowledge, this is the first study analyzing the adaptation of the PTmechanical properties to RT with SWE. Our initial hypothesis that RT would increase PTμ was not confirmed. Also, the VL μ increased significantly, contradicting our previsions based on previous literature, reflecting a faster response from the muscle to RT compared to the tendon´s. This quicker response seen in the muscle can shed a light in the mechanisms leading to tendon injury in environments where muscle force increases too quickly, especially in users of anabolic steroids. Another relevant aspect is that the PT μ oscillations did not presented any correlations with the changes in the VL MT, VL μ, KT or PTT. These findings can help design further researches on the field and build new knowledge about the knee extensor mechanism remodeling process to mechanical overloading.

6. CONCLUSION

The present study showed that an 8-week RT protocol resulted in increasedVL µ but not PT µ, measured by SWE. This research also revealed that the RT applied was effective in promoting quadriceps hypertrophy and strength gains, reflected in increased VL MT and KT, but not in changing PTT. These results reflect that the VL responds morphologically and mechanically faster than the PT to the proposed RT protocol. Further investigation should be conducted with special attention to longer interventions, to possible PT structural in CSA, to corrections reducing the guided waves effect bias and to the muscle-tendon resting state tension environment.PADRONIZAR?

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8. APPENDIX

8.1. WrittenInformedConsent

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - TCLE

Projeto de Pesquisa:ADAPTAÇÃO DAS PROPRIEDADES MECÂNICAS DO TENDÃO PATELAR A UM PROGRAMA DE TREINAMENTO RESISTIDO.

Você está sendo convidado a participar de um estudo observacional, do projeto sobre "ADAPTAÇÃO DAS PROPRIEDADES MECÂNICAS DO TENDÃO PATELAR A UM PROGRAMA DE TREINAMENTO RESISTIDO", de responsabilidade do pesquisador Pietro Mannarino. Todos os participantes estão sendo recrutados pelo corpo médico do Hospital Universitário Clementino Fraga Filho.

Essas informações estão sendo fornecidas para sua participação voluntária neste estudo que visa analisar a adaptação das características do tendão patelare da força de extensão do joelho a um treinamento de musculação, utilizando uma técnica de avaliação não invasiva por ultrassom chamada elastografia SSI (supersonic shearwave imaging) e uma técnica não invasiva de mensuração de força chamada BIODEX.

Todo movimento que o corpo humano realiza é gerado através da transmissão de força dos músculos aos ossos pelos tendões. A elastografia SSI permite avaliar as características dos tecidos de forma não invasiva e quantificar suas adaptações a um programa de musculação progressivo, enquanto o aparelho BIODEX permite quantificar os aumentos de força muscular após um protocolo de musculação.

Primeiramente, será respondido um questionário com suas características e histórico de doenças e realizaremos a ultrassonografia (US) em seu membro inferior apenas para caracterizar os músculos. Se você concordar em participar da nossa pesquisa, você responderá a um questionário, na sua primeira visita, informando-nos suas características e histórico de doenças. Iremos coletar suas informações, como sua altura e peso com uma balança, o comprimento e circunferência da sua coxa com uma trena flexível.

Após este procedimento, você deitará de barriga para cima com o joelho em cima de uma almofada que manterá o joelho semi-dobrado a 30^o, e marcações serão feitas em sua pele, correspondentes às regiões a serem avaliadas pela US. Tais marcações são facilmente removíveis com álcool, procedimento este que será realizado ao fim das medidas. Essas marcações irão servir como locais para que as imagens da SSI (ultrassom) da sua coxa e tendão patelar sejam adquiridas. Não será realizado nenhum procedimento invasivo.

No aparelho BIODEX, solicitaremos que você que realize o máximo de força possível para executar um movimento contra uma resistência. Essa tarefa lhe será solicitada duas vezes, com o intervalo de 60 segundos entre elas, para os movimentos realizados no joelho. Você deverá iniciar e interromper a tarefa conforme o comando verbal do avaliador. Serão calculados os valores máximos da maior força exercida por você. Um alvo com o nível de força será mostrado no monitor do equipamento e você deverá sustentar a força por 10 segundos. Os avaliadores lhe auxiliarão durante a tarefa, encorajando a desempenhar sua força máxima quando solicitada. Seremos cautelosos para que os testes não causem fadiga e qualquer dano devido a pesquisa será de responsabilidade do pesquisador que lhe prestará total assistência.

Os testes serão realizados no laboratório de Análise de Movimento e Fisiologia do Exercício – LAMFE, pelo período de dois dias (antes e depois do protocolo de treinamento), podendo ser solicitado que você retorne em mais um, caso todos os testes não possam ser concluídos no mesmo dia. Sua participação é voluntária e você poderá recusar-se a participar, retornar outro dia (se necessário) e retirar seu consentimento a qualquer momento da pesquisa, sem penalização por isso. Todas as despesas necessárias para a realização deste estudo são de responsabilidade do grupo de pesquisa, não cabendo qualquer custo a você. Seus gastos com qualquer despesa associada à participação na pesquisa, como transporte e alimentação, serão ressarcidos pelos pesquisadores. Serão garantidas todas as informações que você queira, antes, durante e depois do estudo. Ao final, você será convidado a participar do seminário de apresentação dos resultados conclusivos.

Após os testes no aparelho BIODEX e elastografia SSI, você será submetido a um protocolo de musculação durante oito semanas, com uma frequência de duas vezes por semana, totalizando 16 visitas. As sessões serão realizadas no período da manhã entre 8 e 10 horas da manhã na sala 10B12 do Hospital Universitário Clementino Fraga Filho, 10º andar. Inicialmente, uma sessão de familiarização aos exercícios utilizados e teste de cargas será realizada, fato que será repetido ao final do acompanhamento. Todas as sessões de testes, familiarização e musculação serão acompanhadas por dois profissionais de saúde (um médico e um profissional de educação física). Todas as instruções para a prática da atividade, execução dos exercícios e progressão de cargas será feita de perto por esses profissionais de saúde e qualquer dúvida poderá ser sanada.

Caso você venha apresentar fadiga, o teste de força será suspenso imediatamente e só o retomaremos se você julgar-se capaz e/ou os profissionais de saúde envolvidos neste estudo (um médico e um educador físico) garantirem através de avaliação que você encontra-se apto. Estarão presentes no teste apenas um avaliador e um auxiliar e pelo menos um será do mesmo sexo do participante. Este procedimento é para minimizar qualquer constrangimento que você possa apresentar pelo uso da vestimenta necessária para mostrar coxas e panturrilhas, composta por shorts ou bermudas.

Você poderá sentir desconforto ao fazer força máxima no aparelho que medirá sua força assim como durante os dias de treinamento de musculação. Estaremos atentos a sua sensibilidade à dor. O risco de dores musculares assim como a possibilidade de qualquer estiramento muscular ou lesão de tendão durante as sessões de treinamento será controlado com a presença de um ortopedista e um profissional de educação física durante todas as execuções dos exercícios, fornecendo orientações sobre as corretas execuções dos mesmos e supervisionando para imediata interrupção do protocolo diante qualquer desconforto. Você terá garantido o seu direito a buscar indenização po r danos decorrentes da pesquisa conforme previsto pela lei.

Mantemos o compromisso de assegurar seu bem-estar do inicio ao fim do estudo, por isso pedimos que você se comprometa que as informações solicitadas no questionário sejam verdadeiras. Estamos estruturando nossa amostra com participantes de pesquisa livre de qualquer doença, pois nosso objetivo é estudar uma amostra de participantes saudáveis.

Seu nome não será mencionado nem utilizado de maneira alguma em qualquer momento da pesquisa, o que garante o anonimato. O principal benefício deste estudo é proporcionar informações que possam responder questões relacionadas ao comportamento dos tendões do membro inferior em situações de sobrecarga crescente. Espera-se que o conhecimento gerado seja estendido para o bem coletivo. As informações coletadas ficarão arquivadas por 5 anos no computador do laboratório. Após esse período as informações serão deletadas.

O termo será assinado mediante ao esclarecimento de toda e qualquer dúvida, que poderá ser explicada pelo pesquisador Pietro Mannarino no telefone 32158460 ou 998118454 ou através do e-mail mannarino@ufrj.br. O participante e deverá rubricar todas as folhas deste documento com exceção da última página.

Este projeto foi aprovado pelo Comitê de Ética em Pesquisa do Hospital Universitário Clementino Fraga Filho – CEP/HUCFF – Rua Professor Rodolpho Paulo Rocco, nº 255 – Cidade Universitária/ Ilha do Fundão – 7º andar, Ala E, pelo telefone Tel.: 3938-2480 e FAX: 3938-2481, de segunda a sexta, de 8 às 16hs, ou através do e-mail: cep@hucff.ufrj.br. Se você tiver alguma consideração e dúvida sobre ética da pesquisa, entre em contato com o CEP. O Comitê de Ética em Pesquisa é um órgão que controla as questõeséticas das pesquisas na instituição (UFRJ) e tem como uma das principais funções proteger os participantes da pesquisa de qualquer problema

Declaro que concordo em participar da pesquisa . Eu receberei uma via desse Termo de Consentimento Livre e Esclarecido (TCLE) e a outra ficará com o pesquisador responsável por essa pesquisa . Além disso, estou ciente de que eu e o pesquisador responsável deveremos rubricar todas as folhas desse TCLE e assinar na última folha.

____

Participante de Pesquisa

________________________________ Data, ______/______/ ______

Assinatura do Participante de Pesquisa

____

Pesquisador Responsável

________________________________ Data, ______/______/ ______

Assinatura do Pesquisador Responsável

8.2. Project submission to theEthics Committee

Plataforma Brasil MINISTÉRIO DA SAÚDE - Conselho Nacional de Saúde - Comissão Nacional de Ética em Pesquisa – CONEP

FOLHA DE ROSTO PARA PESQUISA ENVOLVENDO SERES HUMANOS

1. Projeto de Pesquisa:
AVALIAÇÃO DA ADAPTAÇÃO DAS PROPRIEDADES MECÂNICAS DO TENDÃO PATELAR A UM PROGRAMA DE TREINAMENTO RESISTIDO

2. Número de Participantes da Pesquisa: 15

3. Área Temática:

4. Área do Conhecimento:
Grande Área 4. Ciências da Saúde

PESQUISADOR RESPONSÁVEL

5. Nome:
Pietro Mannarino

6. CPF:	7. Endereço (Rua, n.º):		
105.221.797-46	GASPAR MAGALHAES JARDIM GUANABARA 130 Casa RIO DE JANEIRO RIO DE JANEIRO 21940120		
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Termo de Compromisso: Declaro que conheço e cumprirei os requisitos da Resolução CNS 466/12 e suas complementares. Comprometo-me a utilizar os materiais e dados coletados exclusivamente para os fins previstos no protocolo e a publicar os resultados sejam eles favoráveis ou não. Aceito as responsabilidades pela condução científica do projeto acima. Tenho ciência que essa folha será anexada ao projeto devidamente assinada por todos os responsáveis e fará parte integrante da documentação do mesmo.

Data: 14 / 05 / 18

Pietro Mannarino
Assinatura

INSTITUIÇÃO PROPONENTE

12. Nome:	13. CNPJ:	14. Unidade/Órgão:
UNIVERSIDADE FEDERAL DO RIO DE	33.663.683/0001-16	COPPE/UFRJ
15. Telefone: 21. 3622.3477	16. Outro Telefone: 21. 3938 8629	

Termo de Compromisso (do responsável pela instituição): Declaro que conheço e cumprirei os requisitos da Resolução CNS 466/12 e suas Complementares e como esta instituição tem condições para o desenvolvimento deste projeto, autorizo sua execução.

Responsável: EDSON HIROKAZU WATANABÉ CPF: 383 506 887-34

Cargo/Função: DIRETOR

Data: 15 / 05 / 2018

Assinatura

Prof. Edson Hirokazu Watanabe
Diretor
COPPE/UFRJ
Matrícula SIAPE - 0360857

COPPE

PATROCINADOR PRINCIPAL

Não se aplica.

8.3. University Hospital Ethics Committee clearance

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1134646.pdf	13/07/2018 16:33:13		Aceito
Folha de Rosto	folha_de_rosto.pdf	13/07/2018 16:03:51	Pietro Mannarino	Aceito
Cronograma	Cronograma.docx	11/07/2018 23:31:46	Pietro Mannarino	Aceito
Parecer Anterior	Carta_Resposta.docx	11/07/2018 23:30:45	Pietro Mannarino	Aceito
Projeto Detalhado / Brochura Investigador	Brochura.docx	11/07/2018 23:27:49	Pietro Mannarino	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Doutorado.docx	11/07/2018 23:27:11	Pietro Mannarino	Aceito
Declaração de Pesquisadores	Carta_apresentacao.docx	28/05/2018 20:09:01	Pietro Mannarino	Aceito
Declaração de Pesquisadores	Carta_apresentacao.pdf	28/05/2018 20:08:50	Pietro Mannarino	Aceito
Outros	Curriculos.docx	28/05/2018 19:44:11	Pietro Mannarino	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 09 de Agosto de 2018

Assinado por:
Carlos Alberto Guimarães
(Coordenador)

8.4. Published research

PLOS ONE

RESEARCH ARTICLE

An 8-week resistance training protocol is effective in adapting quadriceps but not patellar tendon shear modulus measured by Shear Wave Elastography

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Citation: Mannarino P, Matta TTd, Oliveira LFd (2019) An 8-week resistance training protocol is effective in adapting quadriceps but not patellar tendon shear modulus measured by Shear Wave Elastography. PLoS ONE 14(4): e0205782. https://doi.org/10.1371/journal.pone.0205782

Editor: Antoine Nordez, Universite de Nantes, FRANCE

Received: September 28, 2018

Accepted: March 15, 2019

Published: April 16, 2019

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: The author(s) received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: E, Young´s Modulus; KE, knee extension; KT, knee extension torque; MT, muscle thickness; PT, patellar tendon; PTT, patellar tendon

Abstract

Habitual loading and resistance training (RT) can lead to changes in muscle and tendon morphology as well as in its mechanical properties which can be measured by Shear Wave Elastography (SWE) technique. The objective of this study was to analyze the Vastus Lateralis (VL) and patellar tendon (PT) mechanical properties adaptations to an 8-week RT protocol using SWE. We submitted 15 untrained health young men to an 8-week RT directed for knee extensor mechanism. VL and PT shear modulus (μ) were assessed pre and post intervention with SWE. PT thickness (PTT), VL muscle thickness (VL MT) and knee extension torque (KT) were also measure pre and post intervention to ensure the RT efficiency. Significant increases were observed in VL MT and KT (pre = 2.40 ± 0.40 cm and post = 2.63 ± 0.35 cm, p = 0.0111, and pre = 294.66 ± 73.98 Nm and post = 338.93 ± 76.39 Nm, p = 0.005, respectively). The 8-week RT was also effective in promoting VL μ adaptations (pre = 4.87 ± 1.38 kPa and post = 9.08.12 ± 1.86 kPa, p = 0.0105), but not in significantly affecting PT μ (pre = 78.85 ± 7.37 kPa and post = 66.41 ± 7.25 kPa, p = 0.1287) nor PTT (baseline = 0.364 ± 0.053 cm and post = 0.368 ± 0.046 cm, p = 0.71). The present study showed that an 8-week resistance training protocol was effective in adapting VL μ but not PT μ. Further investigation should be conducted with special attention to longer interventions, to possible PT differential individual responsiveness and to the muscle-tendon resting state tension environment.

Introduction

Skeletal muscles act as active and primary motors for the body segments movements [1], while tendons represent an important connective tissue with high resistance to tensile loading, responsible for muscle force transmission to the bone [2]. Both tendon tensile environment and muscle demand levels will determine adaptations in these structures [3,4]. According to the overload environment, tendons can increase resistance and thickness or undergo

Semitendinosus and patellar tendons shear modulus evaluation by supersonic shearwave imaging elastography

C. R. C. Fontenelle[1], P. Mannarino[1,2], F. B. D. O. Ribeiro[1], M. A. Milito[3], A. C. P. Carvalho[3], L. L. Menegaldo[2] and L. F. Oliveira[2]

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This manuscript presents new findings from a research that investigates tendon mechanical properties assessed by supersonic shearwave imaging.

Accepted for publication

Received 14 June 2017;
accepted 3 January 2018

Key words

patellar tendon; semitendinosus tendon; shear modulus; supersonic shear wave imaging; supersonic shearwave imaging; young modulus

Summary

Purpose Shear modulus (μ) is directly correlated to the tissue stiffness and can predict the tendon ultimate force to failure. With the knee extended 0° (K0), semitendinosus tendon (ST) is tensioned while patellar tendon (PT) is relaxed. At 80°, knee flexion (K80) tendons present an opposite stress pattern; however, the relation between ST and PT μ in both situations was not studied yet.

Method We accessed the μ of the ST and PT at 0° and 80° knee flexion by supersonic shear wave imaging (SSI) elastography from 18 healthy males. Relative μ indexes were calculated for relaxed and tensioned conditions.

Result The average μ for ST was $\mu_{ST\text{-}K0} = 197{\cdot}62 \pm 31{\cdot}93$ kPa and $\mu_{ST\text{-}K80} = 77{\cdot}76 \pm 30{\cdot}08$. For TP, values were $\mu_{TP\text{-}K0} = 23{\cdot}45 \pm 5{\cdot}89$ and $\mu_{TP\text{-}K80} = 113{\cdot}92 \pm 57{\cdot}23$ kPa. Relative μ indexes were calculated for relaxed ($IR = \mu_{ST\text{-}K80}/\mu_{TP\text{-}K0}$) and tensioned conditions ($IT = \mu_{ST\text{-}K0}/\mu_{PT\text{-}K80}$). The relative μ indexes were $IR = 3{\cdot}63 \pm 1{\cdot}50$ and $IT = 2{\cdot}00 \pm 0{\cdot}96$ ($P<0{\cdot}05$).

Conclusion Semitendinosus tendon μ was significantly higher than PT μ in both tensioned and relaxed positions. This can predict a higher ultimate force to failure and a less elastic behaviour in ST grafts when compared to PT grafts. This new parameter could aid physicians in graft choice previous to anterior cruciate ligament reconstruction.

Introduction

Knee ligament injury is a growing problem in clinical orthopedics due to the popularization of sport practices. The anterior cruciate ligament injury (ACL) is one of the most studied lesions both because of its prevalence as its acute and long-term consequences. It is still controversial among specialists what is the best graft to be used in the reconstruction. Patellar and semitendinosus tendons are the most popular choices (Liden *et al.*, 2007; Biuk *et al.*, 2015). The factors influencing this decision include the biomechanical properties of each graft frequently addressed through *in vitro* tensile tests (Hamner *et al.*, 1999; Liden *et al.*, 2007; Biuk *et al.*, 2015). The possibility to assess mechanical characteristics of the tendons preoperatively *in vivo* and non-invasively could be a significant advance for clinical decision. The most appropriate graft for a particular subject should be selected based on stiffness and resistance criteria. For a cable-like structure, these properties depend both on material and geometry, namely the longitudinal Young modulus (E), the cross-sectional area and the free graft length.

The early developments of the ultrasound elastographic technique twenty years ago (Ophir *et al.*, 1991) evolved to the real-time analysis of the tissue elasticity with supersonic shear wave imaging (SSI) which can be considered the state of the art (Gennisson *et al.*, 2013). The general principle of SSI relies on the application of high-intensity acoustic force (push) at different tissue depths. It generates shear waves that propagate transversely to the push wave direction. The transducer itself reads these waves in the ultrafast mode (up to 20 MHz), and the system calculates the waves propagation speed and shear modulus (μ). For isotropic elastic media, the shear modulus is related with Young's modulus (or modulus of elasticity, E) that result from *in vitro* tensile tests through the approximation $E \approx 3\mu$ (Royer *et al.*, 2011).

Supersonic shearwave imaging was initially developed for assessing elastic properties of pathological tissues with isotropic characteristics such as breast and liver (Olgun *et al.*, 2014; Park *et al.*, 2015) but has been also applied to study the musculoskeletal system (Shinohara *et al.*, 2010; Bouillard *et al.*, 2011). The SSI data from patellar tendon (PT) have been reported in recent years for pathological and healthy tendons (Peltz *et al.*, 2013; Zhang & Fu, 2013; Ooi *et al.*, 2016);

Clin Physiol Funct Imaging (2017) doi: 10.1111/cpf.12424

Analysis of the correlation between knee extension torque and patellar tendon elastic property

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Accepted for publication
Received 18 August 2016;
accepted 16 February 2017

Key words
elastic properties; knee torque; patellar tendon; supersonic shearwave imaging; Young's modulus

Summary

Purpose Quadriceps strength and patellar tendon (PT) are directly linked and intimately related to daily activities and lower limb function. However, the correlation between knee extension torque (KT) and PT Young's modulus (E) measured directly is still unknown.
Method We used supersonic shearwave imaging (SSI) to evaluate the elastic property of PT in healthy young men and analysed its correlation with KT. Twenty-two healthy young males were included and both knees were examined. The E of the PT in the dominant and non-dominant legs was assessed by SSI elastography. KT in maximal voluntary isometric contraction was measured with an isokinetic dynamometer.
Result No correlations between KT and PT E were observed in dominant or non-dominant side ($P = 0{\cdot}458$ and $0{\cdot}126$, respectively). No significant differences in KT or PT E were observed between both legs ($P = 0{\cdot}096$ and $0{\cdot}722$, respectively). Intra-day ICC was rated good (D1 – $0{\cdot}886$, $P<0{\cdot}001$ and $0{\cdot}88$, $P<0{\cdot}001$) and excellent (D2 – $0{\cdot}928$, $P<0{\cdot}001$ and $0{\cdot}900$, $P<0{\cdot}001$) for both legs. Inter-day ICC was rated moderate for both legs ($0{\cdot}651$, $P = 0{\cdot}016$ and $0{\cdot}630$, $P = 0{\cdot}018$, respectively).
Conclusion No significant correlations were found between KT and PT E, suggesting that quadriceps strength is not an accurate predictor for PT mechanical properties in subjects with no specific training engagement. Habitual loading pattern can play a determinant role in PT mechanical properties, regardless of quadriceps strength. Further investigation on SSI acquisition protocols should be conducted to guarantee higher inter-day ICC values.

Introduction

Tendons are important connective tissue structures, responsible for transmitting muscle forces to produce normal joint motion and mechanical torque (Józsa & Kannus, 1997). Its mechanical properties influence movement patterns and are fundamental to guarantee efficient force transmission but also reduced bone–tendon interface stress. The patellar tendon (PT) deserves particular interest for its function, being responsible for transmitting the quadriceps muscle strength, which is fundamental for lower limb movement, locomotion and normal daily activities (Józsa & Kannus, 1997). Patellar tendon properties have been related to the individual loading environment (Couppe et al., 2008; Westh et al., 2008; Kubo et al., 2010; Seynnes et al., 2013; Zhang et al., 2015), gender and age (Onambele et al., 2007; Westh et al., 2008; Hsiao et al., 2015) and previous existence of any tendon disorder (Zhang et al., 2014; Ooi et al., 2015).

The study of tendons mechanical properties has been of particular interest in the last decades, first performed as an indirect assessment through calculation using B-mode ultrasonography or magnetic resonance imaging (Onambele et al., 2007; Westh et al., 2008; Seynnes et al., 2009, 2013; Svensson et al., 2012) together with dynamometer data. Recently, ultrasound elastography was applied to access PT E *in vivo* (Kot et al., 2012). In that context, supersonic shear imaging (SSI) rises as a valuable tool for PT evaluation (Kot et al., 2012; Zhang & Fu, 2013; Zhang et al., 2014, 2015; Hsiao et al.,

