UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

ESCOLA DE EDUCAÇÃO FÍSICA, FISIOTERAPIA E DANÇA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DO MOVIMENTO HUMANO

Francesca Chaida Sonda

INTRA AND INTER-RATER RELIABILITY OF TRICEPS SURAE MORPHOLOGICAL AND MECHANICAL PROPERTIES IN HEALTHY SUBJECTS

PORTO ALEGRE - RS
2018

Francesca Chaida Sonda

INTRA AND INTER-RATER RELIABILITY OF TRICEPS SURAE MORPHOLOGICAL AND MECHANICAL PROPERTIES IN HEALTHY SUBJECTS

Master's Dissertation presented to the Graduate Program in Human Movement Sciences of the School of Physical Education, Physiotherapy and Dance of the Universidade Federal do Rio Grande do Sul, as requisite for obtaining the title of Master in Human Movement Sciences

Supervisor: Prof. Dr. Marco Aurélio Vaz

Porto Alegre

2018

CIP - Catalogação na Publicação

Sonda, Francesca Chaida
Intra and Inter-rater reliability of triceps surae morphological and mechanical properties in healthy subjects / Francesca Chaida Sonda. -- 2018.
120 f.
Orientador: Marco Aurélio Vaz.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Escola de Educação Física, Programa de Pós-Graduação em Ciências do Movimento Humano, Porto Alegre, BR-RS, 2018.

1. Achilles Tendon. 2. Reliability. 3. Ultrasonography. 4. Tendinous muscle properties. 5. Triceps Surae. I. Vaz, Marco Aurélio, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Francesca Chaida Sonda

INTRA AND INTER-RATER RELIABILITY OF TRICEPS SURAE MORPHOLOGICAL AND MECHANICAL PROPERTIES IN HEALTHY SUBJECTS

Final Degree:

Approved in ....

Examination Board

Prof. Dra. Cláudia Tarragô Candotti

Universidade Federal do Rio Grande do Sul (UFRGS)

Prof. Dr. Bruno Manfredini Baroni

Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA)

Profa. Dra. Rita de Cássia Marqueti Durigan

Universidade de Brasília (UNB)

AGRADECIMENTOS

Ao findar esse processo tão intenso de formação, é maravilhoso poder agradecer a um grupo especial de pessoas e instituições que dele participaram direta e/ou indiretamente. A seu modo e a seu tempo, cada qual contribuiu de forma incontestável para tornar possível o alcance desse sonho.

Agradeço imensamente a minha família, principalmente a minha mãe por representarem “a base do campo”. Em particular, nesse momento, gostaria de agradecê-los por terem me proporcionado uma formação de muita qualidade ao longo de toda a minha trajetória escolar e universitária, além do incentivo, exigência nos estudos e sempre me ensinando a sempre tentar dar o meu melhor. Muito dos êxitos que trago aqui é fruto destes investimentos anteriores. Ao meu pai, apesar de não estar mais presente entre nós, mas por sempre sentir a sua presença ao seu lado.

Agradeço ao meu orientador, Prof. Marco Aurélio Vaz, por todos os ensinamentos, oportunidades, e por ter me incentivado a superar os meus limites. Você é um exemplo de pesquisador/cientista/professor/pessoa que procuro seguir em minha vida!!!

Aos colegas do GPBiC, aos quais se tornaram minha família nestes anos que estou em Porto Alegre. Muito obrigada pela parceria, risadas, conselhos, advertências, estudos e comilanças. Agradeço a Fernanda Metzen, Anna Torresan, Klauber Pompeo, Isabel Paz, Eliane Guadagnin, Matias Frolich, Rodrigo Rodrigues, Emmanuel Rocha, Rodrigo Rabello, Kelli Klein, Bruna Mozzaquatro, Daniele Gomes, Morgana Lunardi, Talita Molinari, Edgar Wagner.

Agradeço imensamente ao professor e amigo, Jeam Marcel Geremia, pelos primeiros ensinamentos e acolhida no grupo ainda quando ingressei na Especialização em Cinesiologia. Obrigada por todas as orientações e os constantes ensinamentos. Te considero um grande mestre, e um exemplo de ser humano, professor e pesquisador.

Agradeço a minha cunhada, Fernanda Brandalise, por ter me acolhido em Porto Alegre antes do ingresso do mestrado oferecendo sua casa para estadia.

Agradeço ao Prof. e amigo, Fábio Juner Lanferdini, por todo o auxílio, conselhos e incentivos nesse período de mestrado.

Agradeço a Profa. e amiga, Eliane Celina Guadanin, por todo o auxílio, e o apoio incondicional na reta final desta etapa.

Agradeço ao Prof. e colega, Emmanuel Rocha, por ser o meu parceiro no grupo do tendão de Aquiles desde quando iniciamos essa jornada.

Agradeço a minha equipe de coleta de dados, Emmanuel Rocha, Eliane Guadagnin e Rodrigo Rabello, por toda a dedicação e empenho nas coletas e análises dos dados.

Agradeço muito aos colegas (Emmanuel Rocha, Rodrigo Rabello, Eliane Guadagnin e Carolina Fritsch) que me auxiliaram na revisão do texto em língua inglesa dessa dissertação. Sabemos que não foi fácil, e acredito que vocês me auxiliaram muito para que eu pudesse evoluir nesse processo! Foi um excelente trabalho de equipe! Muito Obrigada!!!

Agradeço a uma pessoa muito especial em minha formação acadêmica, Profa. e amiga Mônica de Oliveira Melo. No meu primeiro semestre de graduação, você acreditou no meu potencial, me apresentou a pesquisa científica, me proporcionou e ainda continua proporcionando muitas oportunidades. Eu não tenho palavras para te agradecer tudo que você já fez e continua fazendo por mim!!!

Agradeço aos funcionários da ESEFID/UFRGS, em especial do PPGCMH e do LAPEX, por toda a disponibilidade e auxílio.

Agradeço a banca examinadora dessa dissertação, Profs. Claudia Candotti, Bruno Baroni e Rita Durigan, pelas contribuições significativas nas diversas etapas da realização desta pesquisa.

Agradeço as minhas amigas de Caxias do Sul, Jane Ceconello, Solaine Begnini, Daiana Rech, Stephanie de Cesaro e Danielle Dallegrave que sempre me apoiaram muito nestes dois anos e eram minha “fuga” nos tempos livres.

Por fim, agradeço ao meu irmão e parceiro Eduardo que sempre me acompanhou em todas as etapas da minha vida. Você sempre me protegeu, incentivou e me ajudou quando comecei a caminhar sozinha, não ficando bravo quando eu destruía os seus brinquedos, quando tirava minhas dúvidas nas matérias do colégio, e anos mais tarde me ajudando a estudar para as aulas de anatomia e fisiologia da graduação. Nós dois estamos finalizando uma etapa importante de nossas vidas. Eu finalizo o mestrado e você finaliza a residência médica em Infectologia. Com certeza esses dois anos aqui em Porto Alegre se tornaram muito mais leves sabendo que chegaria em casa e teria você ao meu lado.

Dedicada a minha família, na qual sempre priorizou o estudo em minha vida.

RESUMO

O tendão de Aquiles e os músculos flexores plantares têm um papel fundamental em atividades de vida diária como o levantar de uma cadeira, durante a marcha e o salto. Mudanças na sobrecarga mecânica podem levar tanto a adaptações benéficas nas propriedades mecânicas e morfológicas da unidade músculo-tendão do tríceps sural, como após o exercício físico sistematizado, quanto à perda da estrutura e da funcionalidade, como ocorre na ruptura total do tendão de Aquiles e no envelhecimento. A avaliação das propriedades estruturais e mecânicas do tríceps sural é fundamental para que se possa identificar a perda da condição de saúde, assim como também as melhoras decorrentes do processo de recuperação das estruturas musculoesqueléticas durante um programa de reabilitação. Para tanto, é fundamental que a metodologia empregada para a avaliação das propriedades estruturais e mecânicas da unidade músculo-tendínea do tríceps sural seja confiável, e que os resultados não sejam influenciados pelo avaliador (boa reprodutibilidade inter-avaliador) ou pelo dia e horário de avaliação (boa reprodutibilidade intra-avaliador). Entretanto, ainda faltam evidências na literatura referentes à obtenção de variáveis psicométricas relacionadas à avaliação das propriedades mecânicas e morfológicas do tendão de Aquiles e da arquitetura muscular dos flexores plantares em sujeitos saudáveis. Nessa perspectiva, a presente dissertação apresenta três estudos, a fim de atingir os seguintes objetivos: (Estudo 1) verificar a reprodutibilidade intra e inter-avaliador da arquitetura muscular dos flexores plantares em indivíduos saudáveis. (Estudo 2) Verificar a reprodutibilidade intra e inter-avaliador da morfologia tendínea do tendão de Aquiles em indivíduos saudáveis. (Estudo 3) Verificar a reprodutibilidade intra e inter-avaliador das medidas da capacidade de produção de força do tríceps sural. Os três estudos foram realizados no Setor de Plasticidade Neuromuscular do LAPEX da ESEFID-UFRGS. Todos os participantes foram avaliados nos parâmetros de arquitetura muscular dos flexores plantares (comprimento de fascículo, ângulo de penação e espessura muscular) e do tendão de Aquiles (área de seção transversa e comprimento do tendão total livre) por meio de ultrassonografia musculo esquelética. Contrações voluntárias máximas isométricas foram realizadas em um dinamômetro isocinético. As avaliações ocorreram em 2 momentos distintos: dia 1 e dia 2 pelo avaliador 1 (7 dias de intervalo entre as coletas), e posteriormente dia 2 pelos avaliadores 1, 2 e 3 de forma sucessiva. A análise dos dados foi realizada no software SPSS v. 20.0, por meio de estatística descritiva (média, DP - desvio padrão) e inferencial (CCI – Coeficiente de Correlação Intraclasse; EPM – Erro Padrão entre Medidas = $DP\sqrt{(1-CCI)}$; MMD – Mínima Mudança Detectável = 1,96*EPM), e intervalo de confiança. No estudo 1 verificamos uma alta correlação intra e inter-avaliador para espessura muscular, ângulo de penação e comprimento do fascículo dos flexores plantares (CCI>75), e valores baixos de EPM e MMD. No estudo 2, verificamos uma alta correlação intra e inter avaliador para comprimento do tendão total e área de secção transversa (CCI>75). Entretanto, para a variável de tendão livre, a correlação inter avaliador foi pobre (CCI: 0,580). Comparando a experiência dos avaliadores, os avaliadores 1 e 2 obtiveram um CCI de 0,910, enquanto que entre os avaliadores 1 e 3 o CCI foi de 0,340, e entre os avaliadores 2 e 3 o CCI foi de 0,280. Essas correlações muito baixas para a variável do tendão livre podem ser explicadas pela diferente experiência dos avaliadores e pelo tempo de treinamento na obtenção das medidas. No estudo 3, verificamos uma alta correlação intra e inter-avaliador para os valores de torque isométrico em rampa dos músculos flexores plantares (CCI>90), e valores baixos de EPM e MMD. Conclui-se, a partir dos estudos acima, que existe uma alta confiabilidade das medidas de arquitetura muscular e tendínea e de força tanto intra-avaliador quanto inter-avaliador. Além disso, os dados poderão servir como banco de dados de uma condição de saúde para comparação com dados de pacientes que sofreram lesões no tendão de Aquiles. A metodologia também pode ser aplicada na avaliação de pacientes. Entretanto, alguns aprimoramentos nas técnicas utilizadas são necessários a fim de melhorar a reprodutibilidade e reduzir ainda mais os erros de medida.

Palavras-chave: Tendão de Aquiles, flexores plantares, propriedades musculotendíneas, reprodutibilidade intra e inter-avaliador, ultrassonografia.

ABSTRACT

The Achilles tendon and the plantar flexor muscles play a key role in daily life activities such as raising from a chair, during gait and jump. Changes in mechanical overload can lead to both beneficial adaptations in the mechanical and morphological properties of the triceps surae muscle-tendon unit, such as after systemic exercise, as well as after the loss of structure and functionality after total Achilles tendon rupture and aging. The evaluation of the triceps surae structural and mechanical properties is fundamental so that losses in the health condition can be identified, as well as the improvements resulting from the recovery process of the musculoskeletal structures during a rehabilitation program. Therefore, it is fundamental that the methodology used to evaluate the structural and mechanical properties of the triceps surae muscle-tendon unit is reliable, and that the results are not influenced by the evaluator (high inter-rater reliability) or by the day and time of evaluation (high intra-rater reliability). However, there is still a lack of evidence in the literature regarding the psychometric variables related to the evaluation of the mechanical and morphological properties of the Achilles tendon and the plantar flexors muscular architecture in healthy subjects. In this perspective, the present dissertation presents three studies in order to achieve the following objectives: (Study 1) To verify the intra- and inter-rater reliability of the plantar flexors muscle architecture in healthy individuals. (Study 2) To verify intra- and inter-rater rater Achilles tendon morphology evaluation in healthy individuals. (Study 3) To verify intra and inter-rater reliability of the triceps surae isometric strength measurements. The three studies were performed at the Neuromuscular Plasticity Sector of LAPEX of ESEFID-UFRGS. All participants were evaluated in their triceps surae muscle architecture (fascicle length, pennation angle and muscle thickness) and the Achilles tendon (cross-sectional area, tendon length and free tendon length) parameters by means of skeletal muscle ultrasonography. Maximal isometric voluntary contractions were performed on an isokinetic dynamometer. The evaluations occurred in 2 different moments: day 1 and day 2 by evaluator 1 (7-days interval between collections), and on the second day by 2 additional evaluators (2 and 3) in a successive way. Data analysis was performed in SPSS v. 20.0, by means of descriptive statistics (mean, SD - standard deviation) and inferential analysis (ICC - Intraclass Correlation Coefficient - SEM - Standard Error between Measures = DP√ (1 - ICC) MDC – Minimum Detectable Change = 1.96*EPM), and confidence interval analysis. In study 1, we verified a high intra- and inter-rater correlation for muscle thickness, pennation angle and fascicle length (ICC> 75), and low SEM and MDC values. In study 2, we found a high intra- and inter-rater correlation for total tendon length and cross-sectional area (ICC> 75). However, for the free tendon variable, the inter-rater correlation was low (ICC: 0.580). Comparing the evaluators' experience, evaluator 1 and 2 obtained a ICC of 0.910, whereas evaluators 1 and 3 had an ICC of 0.340, while between evaluators 2 and 3 the ICC was 0.280. These very low correlations for the free tendon variable can be explained by the evaluators' experience and by the training of the measurements. In study 3, we verified a high intra- and inter-rater correlation for isometric torque values of the plantar flexor muscles (ICC> 75), and low SEM and MDC values. Based on the above evidences, we can conclude that there is a high intra-rater and inter-rater reliability for muscle architecture, tendon structure and plantar flexor strength. In addition, our data can be used as a data base for the healthy condition for the comparison with patients with Achilles tendon injury. Our methodology can also be used in patients’ evaluation. However, some improvements in the used techniques are necessary to improve reliability and further reduce measurement errors.

Keywords: Achilles tendon, triceps surae, reliability, ultrasonography, tendinous muscle properties.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AT	Achilles tendon
CA	Calcaneus
CB	Calcaneus Bone
CSA	Cross-sectional area
ESEFID	School of Physical Education, Physiotherapy and Dance
FL	Fascicle Length
GRASS	Guidelines for Reporting Reliability and Agreement Studies
ICC	Intraclass correlation coefficient
LAPEX	Laboratório de Pesquisa do Exercício
LG	Lateral gastrocnemius
MDC	Minimum detectable change
MG	Medial gastrocnemius
MRI	Magnetic Resonance Imaging
MT	Muscle Thickness
MTJ	Myotendinous junction
MVIRC	Maximal voluntary isometric ramping contractions
PA	Pennation Angle
SD	Standard Deviation
SO	Soleus
TL	Tendon Length
UFRGS	Universidade Federal do Rio Grande do Sul
US	Ultrasound

CONTENTS

PREFACE

During the rehabilitation process, patients need to be evaluated and reevaluated in order to quantify changes and verify if there is clinical, structural or functional improvements. Certain measurements have a great value for clinicians when making decisions in clinical research. However, this value depends on if they can rely on the measured data as an accurate and significant indicator of a behavior pattern or a change. Therefore, the measurements must be consistent and error-free.

The musculoskeletal rehabilitation clinical practice depends on precise biomechanical evaluations, with measurements that are not dependent on the rater who obtains the measurements and neither on the evaluation day. The triceps surae morphological and mechanical properties are widely evaluated to obtain information that allows clinicians to determine the presence of losses suffered as a result of a traumatic event, a degenerative disease or an aging process, as well as the return to a healthy condition.

However, systematic studies that evaluate the triceps surae, providing precise measurements and allowing us to determine, from the results obtained, the procedures to be adopted in the evaluation and treatment of patients who have ruptured the Achilles tendon, are still lacking. Thus, studies that quantify psychometric variables, evaluating the intra and inter-rater reliability of these measurements are necessary. This makes it possible to determine if the data obtained in the musculoskeletal evaluations are influenced by the day of data collection and/or by the rater performing the evaluations.

To improve the evaluations' quality, the adoption of a systematic and concise methodology for evaluating the structure of the plantar flexor muscles and of the Achilles tendon, as well as the mechanical and morphological properties of the triceps surae, is necessary. In this way, strategies for the prevention of musculoskeletal injuries are proposed and the recovery process from rehabilitation programs is improved.

Based on the above ideas, the present dissertation was divided into three chapters, developed at the Neuromuscular Plasticity sector of the Exercise Research Laboratory (LAPEX) of the School of Physical Education, Physiotherapy and Dance (ESEFID) of the Federal University of Rio Grande do Sul (UFRGS).

The first chapter shows a literature review on the basic concepts of muscle and tendinous tissue, as well as the main characteristics of the psychometric variables. The second chapter shows a study of intra- and inter-rater reliability of the plantar flexors muscle architecture parameters obtained by ultrasonography in healthy individuals. The third chapter shows an intra- and inter-rater reproducibility study of the Achilles tendon morphological properties in healthy individuals. The last chapter shows a short communication on the intra and inter-rater reliability of the triceps surae isometric strength in healthy individuals.

The data obtained in these three studies will also serve to compose a database of the musculotendinouds unit, which may provide normative data for healthy young individuals. It will also allow us to compare data from Achilles tendon rupture patients, and who today are considered "healthy" after being discharged from the physicians, with our normative data. This will allow us to better identifying the losses resulting from the injury and to indicate the path for the return to a healthy condition.

CHAPTER 1

Literature Review – Contextualization

1.1 The Triceps Surae Muscle

Muscles produce the force that is transmitted via connective tissue to the skeletal system to propel our movement (FINNI, 2006). Our daily living activities and our ability to move are only possible due to the greatest and strongest tendon of the human body, the Achilles tendon (MAFFULLI and ALMEKINDERS, 2007). It consists of the continuation of the connective tissue (endomysium, perimysium and epimysium) of the distal portion of the gastrocnemius and soleus muscles. In addition, it has the function of transmitting the force of these muscles to the posterior portion of the calcaneus, determining the ankle's plantar flexion movement (MAFFULLI, 1999).

As the name indicates, the triceps surae muscle consist of three parts: the gastrocnemius muscle, containing two superficial heads, and the soleus muscle containing the deep head (MAFFULLI and ALMEKINDERS, 2007). Gastrocnemius muscles originate in the femur condyles, and their contributions to the tendon begin with a broad aponeurosis that starts below these muscles (Figure 1.1 and 1.2). The gastrocnemius muscle is two headed, originating from the medial and lateral femur epicondyles, making the surae muscle bi-articular: acting in the knee and the ankle joint (BARFOD, 2014).

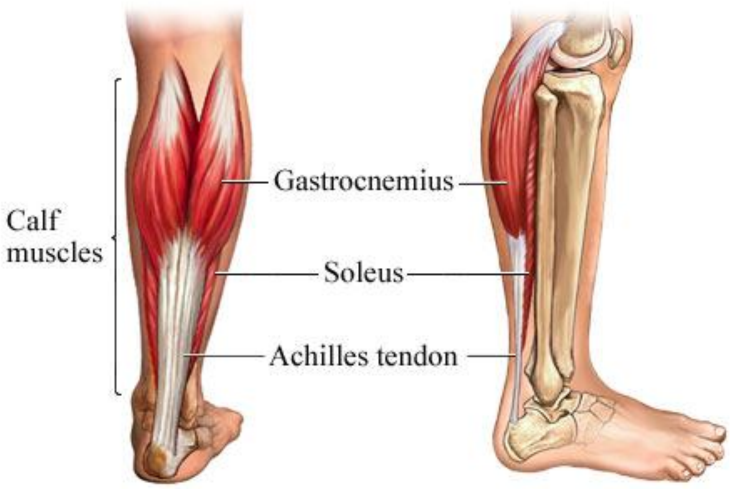


Figure 1.1 The Achilles tendon and the triceps surae muscle anatomy. Source: Barfod, 2014.

The soleus muscle originates in the tibia and the fibula, and its contribution to the tendon is thick but short (MAFFULLI and ALMEKINDERS, 2007). In addition, the soleus muscle inserts itself into a superficial tendon blade that attaches to the tendon blade of the gastrocnemius muscle in the middle of the calf to form the Achilles tendon (BARFOD 2014). The Achilles tendon and the plantar flexor muscles play a fundamental role in getting up from a chair, in gait and in jumping, and therefore, are subjected to great forces, both internal and external, in the propulsion phase of all these daily living activities (MAFFULLI and ALMEKINDERS, 2007).

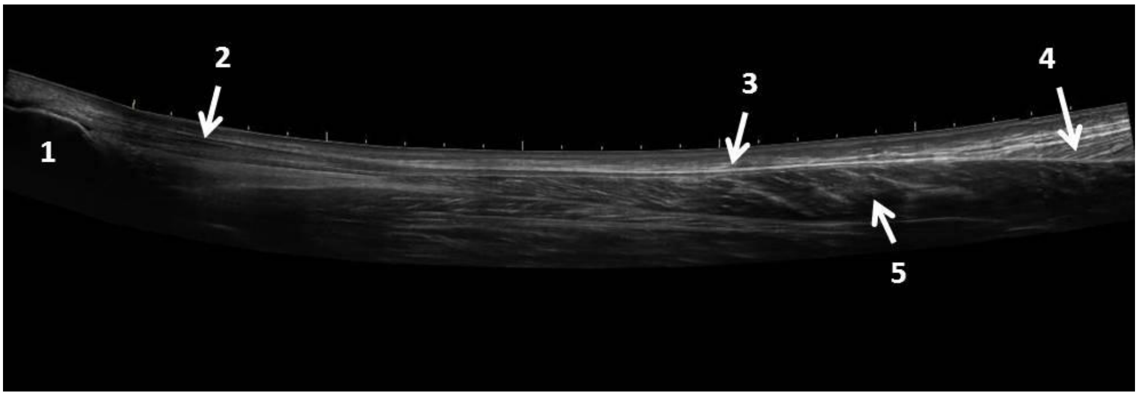


Figure 1.2: A panoramic ultrasound picture of the calf: 1) Calcaneus, 2) The Achilles tendon, 3) the convergence of the tendon sheets of the soleus muscle, 4) the gastrocnemius muscle, and 5) the soleus muscle. Source: Barfod, 2014.

1.2 Achilles Tendon

Tendons are responsible for transmitting forces from muscles to bones, a fundamental role for movement production (NIGG and HERZOG, 1999). They are defined as constituent elements of skeletal muscles, and are formed by a dense and regular fibrous connective tissue, where collagen fibers are organized in bundles (BJUR et al., 2010). They are composed mainly by highly organized collagen bundles, with 95% of type I collagen (MAFFULLI, 1999a, b). Collagen is what ensures the tensile strength and the extensibility of the tendon’s tissue (SHARMA and MAFFULLI, 2005). In addition to collagen, tendons are made up of a small amount of elastin, proteoglycans and glycoproteins (SHARMA and MAFFULLI, 2005; DORAL et al., 2010).

Tendons have a wide variety of shapes and sizes, depending on the morphological, physiological and mechanical characteristics of the muscles and bones to which they are connected (NIGG and HERZOG, 1999). They range from broad sheets to highly elastic cables, such as those of the Achilles' tendon. Because of their structural roles, injuries to tendons are extremely common and often debilitating. Thus, a fundamental question in musculoskeletal biology is how these connective tissue structures develop in the correct locations and acquire the strength necessary to translate contractions of muscles into skeletal movements (SUBRAMANIAN and SCHILLING).

The Achilles tendon (or calcaneus tendon) is the strongest and thickest tendon in the body, and has the function of attaching the triceps surae to the calcaneus. This is characteristic of the human anatomy, and it has been suggested that this tendon has helped to shape human evolution (BRAMBLE and LIEBERMAN, 2004). During running, the Achilles tendon transmits forces to the foot that are approximately seven times the body weight. This represents an enormous increase on the forces that act during standing (which are roughly half the body weight) (MAFFULLI and ALMEKINDERS, 2007). Typically, Achilles tendon is glistening and pearly-white in color, and may take the shape of a broad sheet (NIGG and HERZOG, 1999).

1.3 Tendon Architecture

Usually, tendons consist of an external tendon, which is typically referred to as tendon, and an internal tendon, which is typically referred to as aponeurosis. The external tendon connects the muscle to bone and the aponeurosis provides the attachment area for the muscle fibres (NIGG and HERZOG, 1999).

The Achilles tendon is built of collagen molecules in a complex matrix of left and right turned helices bound together by proteoglycans. Type I collagen constitutes 95% of the total collagen. The remaining 5% consists of type III and V, mainly located on the enthesis and the epitendon (SILVER et al., 2003). The tendon is a hierarchical structure composed of collagen molecules, fibrils, fiber bundles, fascicles and tendon units that run parallel to the tendon's axis (Figure 1.3). Fibers and fascicles are enclosed by the epitendon, which is a fine, loose connective tissue sheath containing the vascular, lymphatic, and nerve supplies to the tendon. The dominant cell type is the fibroblast (tenoblasts and tenocytes), which align in rows between collagen fiber bundles and produce the collagen matrix (WANG et al., 2006). These fibroblasts are embedded in a unique extracellular matrix (ECM) that is composed mainly of collagen fibril arrays capable of withstanding incredibly strong tensile forces (SUBRAMANIAN and SCHILLING).

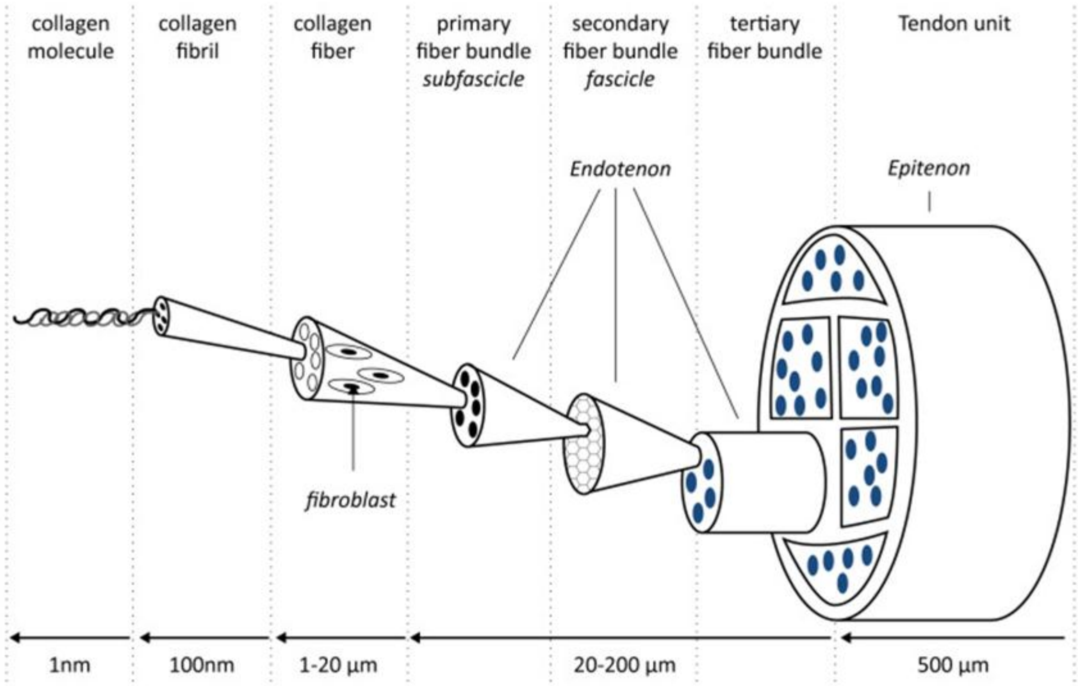


Figure 1.3: A schematic drawing of the tendon as a multi-unit hierarchical structure (modified from Wang et al., 2006).

The frequency, duration, and/or magnitude of tendon forces can change dramatically in response to changes in physical activity and muscle strength, as it occurs after a period of strength training (URLANDO and HAWKINGS, 2007; DUCLAY et al., 2009; SEYNNES et al., 2009), or immobilization due to Achilles tendon rupture (KANNUS et al., 1997). Similarly, the aging process alters the mechanical overload (reduction of mobility) and structure (loss of structure) of the triceps surae (KARAMANIDIS and ARAMPATZIS, 2005; STENROTH et al., 2012).

These changes lead to adaptations in the musculotendinous' mechanical and morphological properties, and may lead to an improvement in the condition of the musculotendinous unit (as in the case of systematized physical activity) or to a loss of health status (as in the case of tendon rupture and aging). (STENROTH et al., 2012; GEREMIA et al., 2015; GEREMIA et al., 2018). Therefore, understanding the morphological and mechanical properties of the triceps muscle-tendon unit is fundamental to be able to identify both the health condition and the improvement during the rehabilitation process and/or worsening of the condition in the case of tendinopathies, immobilization and aging.

1.4 Achilles Tendon Mechanical Properties

In vivo methods allow for longitudinal investigations that could address important functional tissues relations. The identification of effective training regimes for enhancing the tendon's mechanical properties, and the identification of the immobilization duration that starts inducing the tendon properties deterioration are examples of the importance of these longitudinal studies.

The tendons mechanical properties can be evaluated either passively (with the plantar flexors relaxed) or actively (with the plantar flexors contracted). When evaluated passively, ankle joint movements are produced involuntarily, and the tendon deformation is obtained by ultrasonography (US). The passive force for stretching resistance, generated by the tendon and other soft tissues, is measured with an isokinetic dynamometer, which imposes an angular movement to the ankle at a constant angular velocity (ABELLANEDA et al., 2009; MIZUNO et al., 2011; NAKAMURA et al., 2011).

On the other hand, mechanical tendon properties, when measured actively, are obtained during maximal contraction (MAGNUSSON et al., 2001; GEREMIA et al., 2015; GEREMIA et al., 2018). According to Maganaris et al. (2008), the in vivo method is based on real-time ultrasound scanning of a reference point along the muscle-tendon unit during an isometric contraction-relaxation cycle. The muscle forces generated by activation are measurable by dynamometry, causing a longitudinal tendon deformation.

During the plantar flexors' maximal isometric contractions, the antagonist dorsiflexors musculature is also activated (co-contraction), which reduces the maximum force generated by the plantar flexors. In order to correct this problem, it is necessary to quantify the force of the antagonists and to add this to the agonists' force, in order to identify the plantar flexors capacity of maximal force generation. Studies have performed this correction of the agonist torque through the estimation of the antagonist torque (MADEMELI and ARAMPATZIS et al., 2005; ARYA and KULIG, 2010). This estimation is carried out considering a direct relation between muscle electric activation and torque (ARYA and KULIG, 2010), which allows researchers to estimate the torque generated by the antagonist muscle during the maximal test of the agonist muscle. The estimated torque was obtained through the relationship between different levels of submaximal force and corresponding surface electromyography of the antagonist muscles. After correction of the agonist torque, the longitudinal tendinous deformation is measured as a function of the applied muscular force.

From these tests, force-elongation and strain-strain relationships can be obtained. These relationships demonstrate absolute and relative mechanical behavior (SHARMA AND MAFFULLI, 2005; MAFFULLI and ALMEKINDERS, 2007).

The force-elongation relationship quantifies the absolute deformation occurring in the tendon as a function of an applied force (Figure 1.4). The shape of the force-strain curve may be different between different species and between different individuals of the same species due to differences in tendon dimensions. Thus, the normalization of the force-elongation relation by the dimensions of the tendon generates the stress-strain relationship (Figure 1.4), which allows comparisons of the relative mechanical behavior between different species and between different individuals (SHARMA and MAFFULLI, 2005).

Stress is obtained by normalizing the force applied to the tendon by the anatomical cross-sectional area (ACSA) of this structure (force/ACSA). Strain is obtained by normalizing the variation of the tendon length (TL) (subtraction between the final TL and the initial TL) by the tendon initial length. From these relationships we can calculate the tendon stiffness and modulus of elasticity (also known as Young's modulus) (MADEMELI and ARAMPATZIS, 2005).

The slope of the curve represents the tendon stiffness. It is calculated from the force-elongation relationship, and is considered a measure of the entire tendinous structure performance (ARAMPATZIS et al., 2007). The modulus of elasticity is obtained through the stress-strain relationship, and reflects the material properties of a tendon. The stiffness and Young's modulus are obtained from the force-elongation and strain-strain curves' slope, respectively, in the final phase of this exponential curve, that is, in the linear part of the curve.

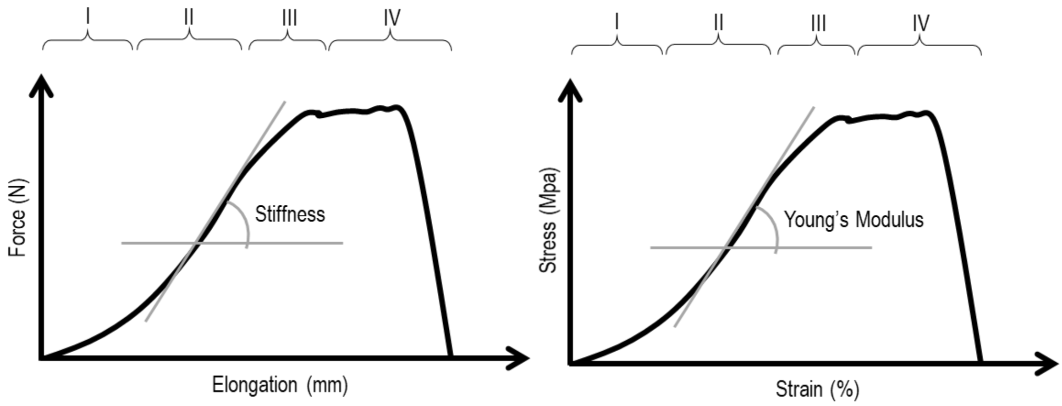


Figure 1.4. Left = Force-elongation relationship; Right = Stress-strain relationship. The figure shows four regions in each curve. I: represents the toe region, where the wavy collagen fibers are stretched with increasing force or stress; II: represents the linear region, where collagen fibers deformation increases linearly with increasing tension; III: represents the plastic region, where there is fiber microdamage; IV: is the region where tissue failure occurs. Source: Adapted from MAGANARIS et al. (2007).

The Achilles tendon is remarkably strong and can withstand stresses that by far exceed those transmitted during daily activities and sports. It has been estimated that the peak force transmitted through the Achilles tendon during running is 9 kN, which is equivalent to 12.5 times the body weight (KOMI, 1992). Our knowledge on biomechanical properties of tendons is mainly derived from animal models and cadaveric studies (BOJSEN, 1996). A typical tendon stress-strain curve is seen in figure 1.5.

The first 2% of elongation represents the stretching-out of the collagen fibers (WANG et al., 2006). As all fibers become stretched, the stress-strain curve becomes linear until the tendon starts failing and microscopic tearing occurs. Beyond an 8-10% strain, macroscopic failure occurs.

These two mechanical tendon properties are important because they are directly related to the Achilles tendon mechanical resistance to stretching.

Moreover, these relationships, when evaluated at the submaximal level and in a cyclic way, allow us to evaluate the storage and return of kinetic strain energy by means of the hysteresis area of the force-elongation and stress-strain curves. Understanding tendinous hysteresis (Figure 1.6) is also important because submaximal stretching forces are also applied to the Achilles tendon during cyclic activities of daily living, such as walking, running and jumping. If the kinetic energy absorbed by the tendon is not returned to the environment, it is stored in the tendon (MAFFULLI and ALMEKINDERS, 2007). The area under the loading stress-strain curve represents the tendons ability to store energy. This residual energy can accumulate along the submaximal cyclic exercises, leading to micro-ruptures or even macroruptures of the tendinous structure. Tendon stiffness and modulus of elasticity may increase due to changes in the material properties of the tendon or changes in tendon morphological properties, such as increased tendon CSA and collagen content (ARAMPATZIS et al. 2007; BOHM et al., 2015).

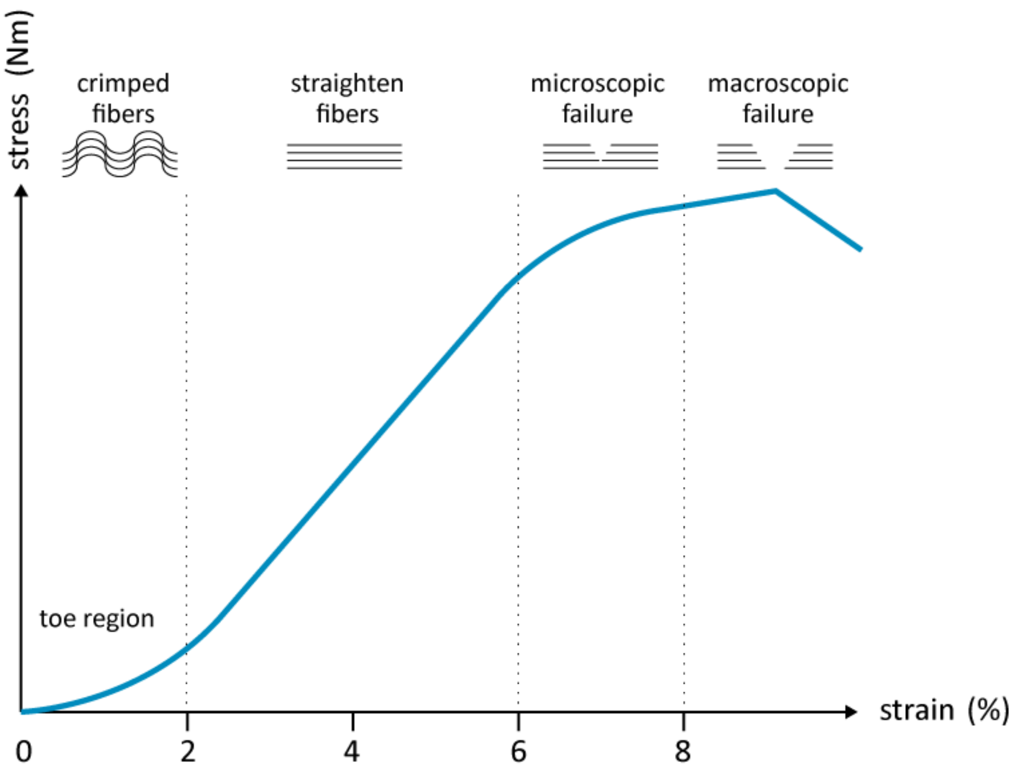


Figure 1.5. A schematic drawing of the stress-strain curve for the Achilles tendon (modified from Wang et al., 2006).

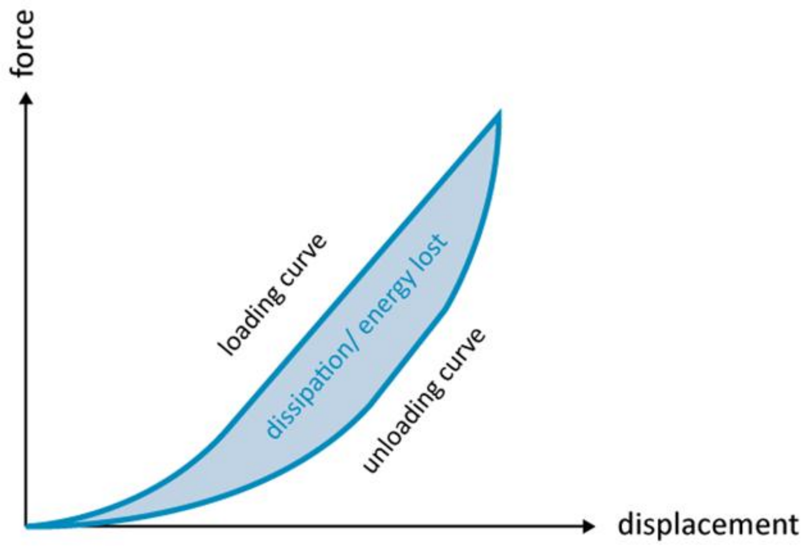


Figure 1.6. A schematic drawing of energy absorption in a tendon and energy lost during the coil-recoil process (modified from Wang et al., 2006).

1.5 Achilles Tendon and Plantar Flexors Muscles Morphological Properties

The mechanical properties of the Achilles tendon are obtained through the relation between the tendon's capacity to resist to a force applied to it and of how much it deforms as a consequence of this tensional force. Therefore, the determination of these properties depends on their structural characteristics, as well as on the measurements of neuromuscular parameters (ARYA and KULIG, 2010).

Although poorly used in clinical practice, tendon morphological parameters can be used to determine the adaptive capacity of the tendon when subjected to changes in the mechanical environment or in important clinical evaluations, such as in the detection of tendinopathies and after tendon rupture and surgical reconstruction. Considering that tendinopathies (ARYA and KULIG, 2010) and tendinous ruptures (GEREMIA et al., 2015) may reduce or even increase the tendon morphological properties (CSA and TL), these also cause changes in the tendon's stiffness and modulus of elasticity (FOURE et al., 2009; GEREMIA et al., 2018).

For example, the inflammatory process due to tendinopathy may cause a temporary increase of the tendon CSA due to edema (ARYA and KULIG, 2010).

This increase in the tendon anatomical structure, however, is not accompanied by an increase in extracellular matrix and fibrous content. In the case of tendon ruptures, CSA may increase more than 100% due to the formation of tendon callus (MAFFULLI and ALMEKINDERS, 2007). This callus is formed by the fimbriae of the two tendinous stumps that are joined by means of a surgical reconstruction suture (MAFFULLI and ALMEKINDERS, 2007; GEREMIA et al., 2015). The objective of this callus is to increase the contact area of the tendon stumps and to provide a greater interaction between the collagen fibers. The increase of the contact area between the two irregular surfaces allows the establishment of tensile forces that generate a shear stress, increasing the resistance capacity of the recovering tendon (BOHM et al., 2015). Despite the Achilles tendon CSA increase immediately after the surgical process, this greater area is not related to an immediate increase in the tendon mechanical properties. Therefore, these tendon structural changes have functional implications, such as reduction in the tendon's ability to transmit force to the bones to which it is inserted, thereby reducing its ability to generate joint movement.

Tendon recovery occurs through a reduction of tendon CSA and an increased deposition of type 1 collagen, instead of type 3 collagen, initially deposited during tendon callus formation (BOHM et al., 2015). In other words, there is a reorganization of the tendon and extracellular matrix structure, during which tissue in excess needs to be removed and more resistant collagen fibers need to be deposited at the site of the tendon callus. The parallel reorganization of the collagen fibers, an organizational characteristic of the tendon structure, also returns its mechanical resistance to the tensile forces (WANG et al., 2012). This structural remodeling depends exactly on the mechanical overload applied to the tendon during the rehabilitation process. Mechanotransduction, by definition, is the mechanism by which cells convert mechanical stimuli into cellular responses due to a variety of mechanical loads (DUNN and OLMEDO, 2016). Therefore, measuring structural changes in the tendon before, during and after the rehabilitation process enables us to have objective measures of the structural adaptations of the Achilles tendon, and should be used as a routine in clinical practice, which unfortunately does not occur.

Different methods to measure Achilles tendon morphology properties have been developed (REES et al., 2008; ARYA and KULIG, 2010, GEREMIA et al., 2015). The methods can be divided into four groups depending on modality: radiographs, ultrasonography (US), Magnetic Resonance Imaging (MRI) and clinical evaluation. US has been established as an important and cost-effective tool in the diagnosis of tendon problems.

Rees et al. (2008) developed a method for measuring tendon length by US. The AT length was defined as the distance between the medial gastrocnemius muscle tendon junction (MTJ) (tendon origin) and the tendon insertion (at the calcaneus) (Figure 1.7).

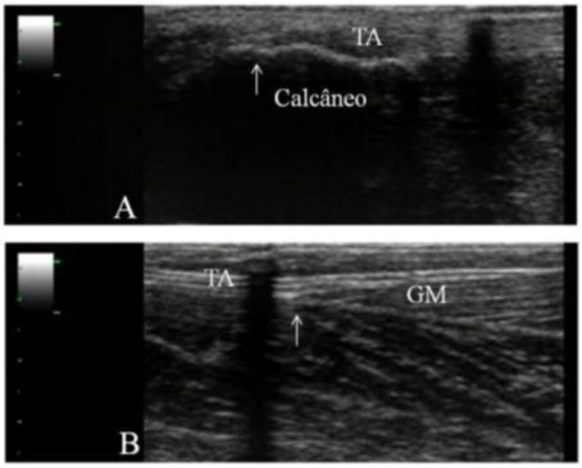


Figure 1.7. Locations for obtaining the necessary parameters to measure the Achilles tendon (TA) length. The arrows indicate the most distal AT insertion in the calcaneus bone (A) and the most proximal insertion at the myotendinous junction of the medial gastrocnemius (GM) muscle (B). Source: Neuromuscular Plasticity Lab at the Exercise Research Laboratory of the Federal University of Rio Grande do Sul.

However, technological advances have improved tendon length measurements, with the development of panoramic imaging (BARFOD et al., 2018) for evaluating the Achilles tendon free part. The length of the Achilles tendon free part was defined as the distance between the most proximal point of the posterior border of the calcaneus and the distal tip of the soleus muscle-tendon junction. The measurement is carried out in two steps: first, the anatomical landmarks are identified and marked on the skin, and then, the distance between them is measured on the skin with a measuring tape (BARFOD et al., 2018; Figure 1.8).

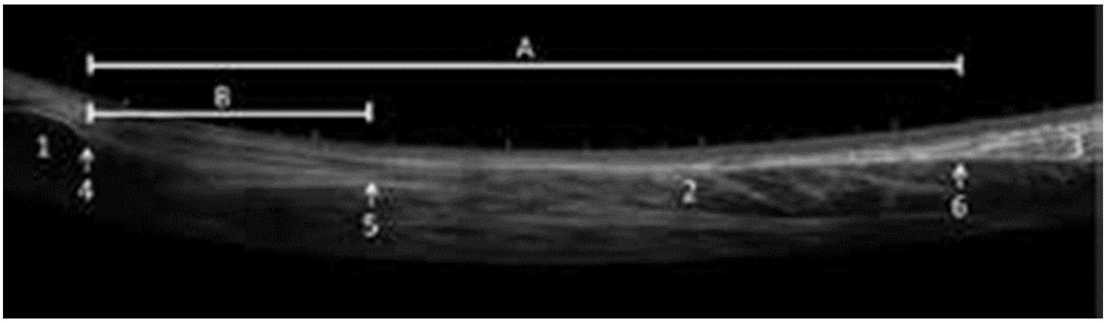


Fig. 1.8 Panoramic ultrasound picture showing the posterior part of the calf. The distance from the calcaneus to the medial head of the gastrocnemius muscle (A). The free part of the Achilles tendon (B). The calcaneus (1), the soleus muscle (2), the medial head of the gastrocnemius muscle (3), the posterior-superior corner of the calcaneus (4), the distal tip of the soleus muscle (5), and the distal tip of the medial head of the gastrocnemius muscle (6). Source: Barfod et al., (2018).

US is the most frequently used imaging method to assess the tendons cross-sectional area (CSA) (PIERRE-JEROME et al., 2010). To obtain CSA measurements, the US transducer is placed perpendicular to the tendon. Three transverse images are taken at distances of 2, 4, and 6 cm proximal to the tendon insertion on the calcaneus (ARYA AND KULIG, 2010; GEREMIA et al., 2018). Another way of performing the tendon length measurement is to obtain the image at positions of 25% (proximal), 50% (middle) and 75% (distal) of the tendon's length (0% refers to the origin) (Figure 1.9).

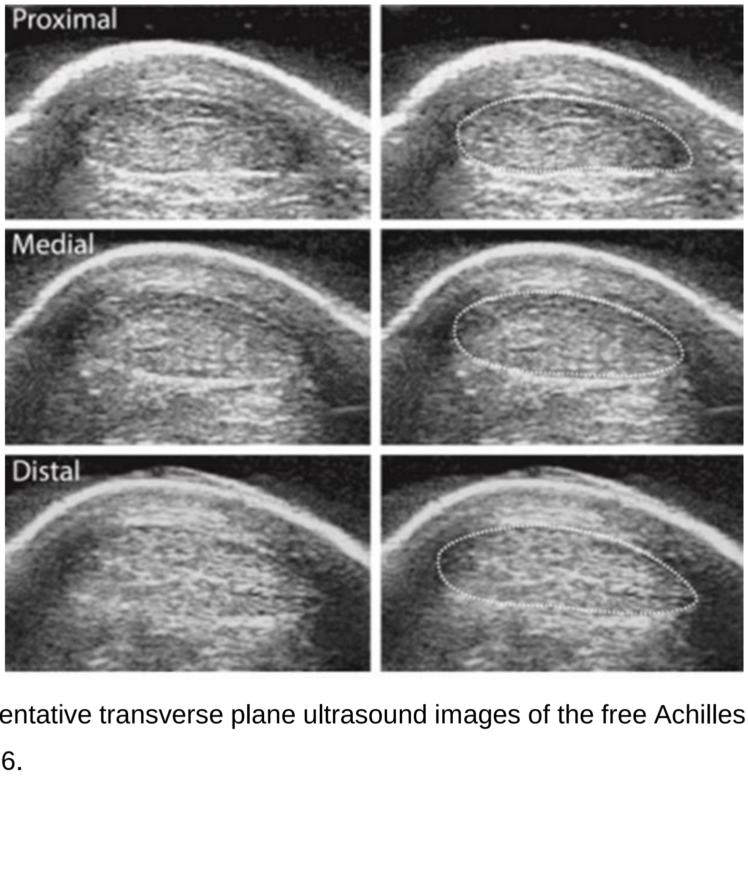


Fig. 1.9. Representative transverse plane ultrasound images of the free Achilles tendon. Source: Bohm et al., 2016.

Similar to the tendon tissue, skeletal muscle is also a tissue highly adaptable to the mechanical and functional demands imposed on it (FRASSON and VAZ 2000), with these adaptations attributed to muscular morphological changes (HERZOG et al., 1991). These adaptations occur due to the high plasticity of the muscular tissue, which can present gains or losses of contractile tissue according to the load variation to which the structure is submitted (NARICI, 1999). These adaptations in the skeletal muscle morphology may alter the muscular architecture (DUCLAY et al., 2009; REEVES et al., 2009; BARONI et al., 2013).

Muscular architecture is understood as the fibers' geometric arrangement in relation to the muscle's line of action of force production (LIEBER and FRIDEN, 2000). It is possible to identify the muscle's functional capacity, since the muscular force production capacity is estimated through the evaluation of different parameters (WARD et al., 2009). The muscle fibers arrangement has a direct relation to the muscle mechanical properties (force-length relationship and force-velocity relationship), which allows us to understand how the muscle structure behaves when generating force (WARD et al., 2009). Increases or reductions in the architecture alter the muscle's ability to generate force in relation to different muscle lengths and different shortening speeds.

These morphological and mechanical properties of the Achilles tendon and the triceps surae muscle have been widely studied and evaluated through the use of US technique (ABELLANEDA et al., 2009; NAKAMURA et al., 2011).

Among the main parameters of muscular architecture are the fascicle length (FL), the pennation angle (PA) and the muscle thickness (MT) (FUKUNAGA et al., 1997, NARICI and CERRETELLI, 1999; BLAZEVICH et al., 2006; BARONI, et al., 2013). FL is defined as the length of the fascicular trajectory between the insertion of the fascicle in the superficial and deep aponeuroses of the muscles (Figure 1.10). The angle originated between the muscle deep aponeurosis and the muscle fascicles line of insertion is considered as the PA (Figure 1.10). The distance between the deep and superficial aponeuroses determines MT (Figure 1.11) (BARONI et al., 2013).

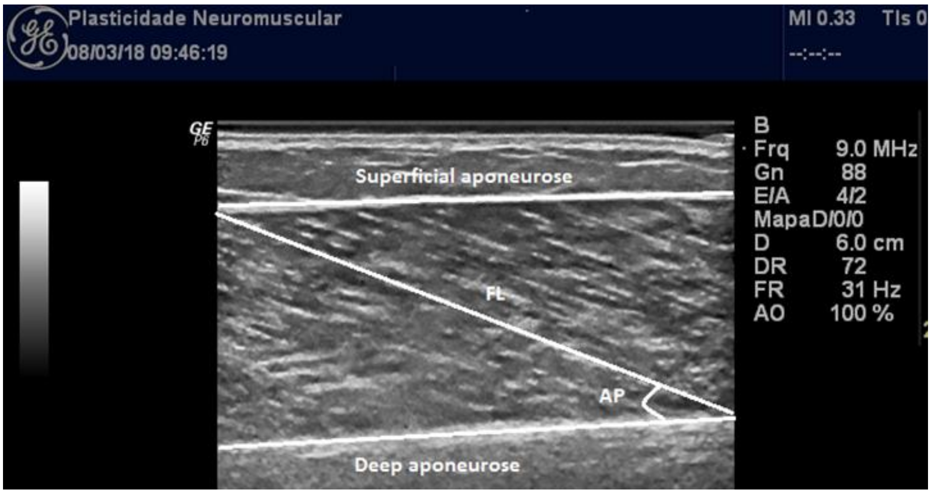


Figure 1.10. Measurement of medial gastrocnemius muscle fascicle length (FL) and pennation angle (AP). Source: Neuromuscular Plasticity Lab at the Exercise Research Laboratory of the Federal University of Rio Grande do Sul.

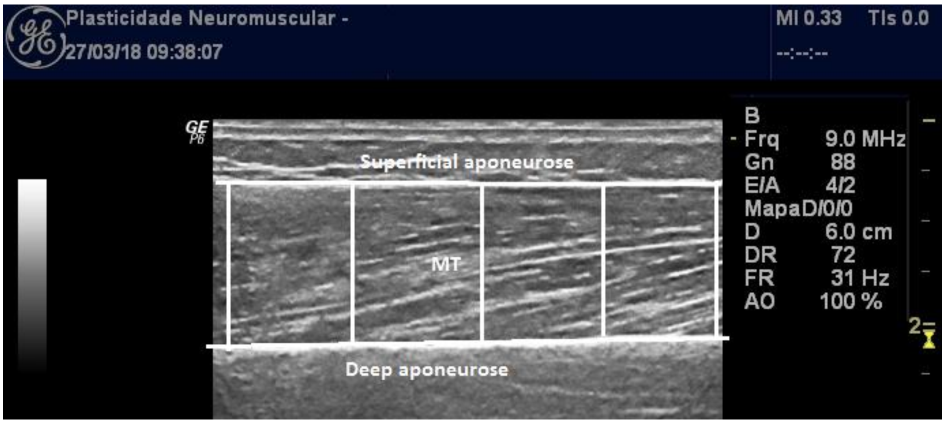


Figure 1.11. Measure of soleus muscle muscle thickness (MT). Source: Neuromuscular Plasticity Lab at the Exercise Research Laboratory of the Federal University of Rio Grande do Sul.

1.6 Musculoskeletal Ultrasonography (US)

Ultrasonography (US) is a non-invasive technique that, although not as accurate as Magnetic Resonance Imaging (MRI), has some advantages: it does not emit ionizing radiation, it has a lower cost of use and it allows static and dynamic viewing of muscle architecture with real-time results, making it more functional than MRI (GILES et al., 2015). The images are formed from echoes that return from the tissues to the probe, which are analyzed by the US device's software and transformed into an image (KAWAKAMI et al., 1993).

Evidence shows that it is a rater dependent evaluation technique (MENDIS et al., 2010), which suggests that a large volume of practice in obtaining the measurements is fundamental and necessary before its use in clinical practice. In addition, the degree of reliability of its measurements depends on a rigid protocol and knowledge of the analyzed structures.

US has been used to investigate the structure of a variety of muscles and to answer a number of research questions (WHITTAKER et al, 2006). Authors affirm that several studies have evaluated the reliability of these measures and presented, in general, good to excellent reliability, considering repeated measures with the individual at rest and measurements of morphological alterations with individuals performing dynamic tasks (WHITTAKER et al., 2006; EKIZOS et al., 2013).

1.7 Triceps Surae Strength Production Capacity

Isokinetic dynamometry has been widely used to assess the capacity of muscular force production (BALTZOPOULOS and BRODIE, 1989). One of the great advantages of isokinetic dynamometry is that it allows the assessment of the maximum (or submaximal) capacity of muscle strength throughout the articular range of motion at a constant velocity (IMPELLIZERI et al., 2008). In addition, it provides an accurate assessment of the torque, work and power of specific muscle groups (IMPELLIZERI et al., 2008). For this reason, it has been considered as the gold standard in the evaluation of musculoskeletal strength production capacity (ALVARES et al., 2015). Therefore, reproducible measurements of the maximum active and passive plantar flexors torque are fundamental so that the mechanical properties and changes of these properties can be determined.

1.8 Morphological and Mechanical Properties in Healthy Subjects

Several studies have evaluated the plantar flexor muscles architecture and the Achilles tendon morphological and mechanical properties in healthy individuals, before and after a period of intervention and/or training, or comparing the values of healthy individuals with those of patients who had ruptured the Achilles tendon or suffered from tendinopathies. The focus of the present study is the evaluation of these properties in healthy subjects. Table 1.1 shows a summary of the studies' results that evaluated these measurements in healthy subjects only. The comparison of the morphological properties measurements between the studies shows that they present similar values. However, it is interesting to note that, despite the complementarity of the structural and mechanical properties of the muscle-tendon unit, few studies have evaluated muscle and tendon morphology jointly.

Table 1.1 Studies evaluating the Achilles tendon mechanical properties of healthy individuals.

	Magnusson et al., 2001	**Duclay et al., 2009**	**Arya e Kulig, 2010.**	**Morrisey et al., 2011**	**Magnusson et al., 2011**	**Geremia et al., 2015**	**Geremia et al., 2018**
Mechanical Properties							
Force (N)	3171.0±3255.0	3000,0	2258.2±26.0	NE	1924±229	3117.8±604.0	2749.5±491.1
Elong. (mm)	13.9±10.7	21,2	11.0±0.9	NE	2.9±0.6	15.2±2.6	17.1±2.6
Stiffness (N/m)	486.0±46.7	200,0	375.3±6	209.0±7.3	262±53.4	210.7±49.4	206.6±51.9
Stress (MPa)	40.3±8,6	NE	40.3±8.6	NE	29±3	53.5±10.6	43.6±7.4
Strain (%)	4.4	NE	4.4±0.3	NE	4.2±1.1	6.6±1.4	7.9±1.4
YM (MPa)	1474.0	NE	1671.0±2.0	579.0±29.2	2000±0.4	885.1±214.1	706.8±19.8

Elong.: Elongation. MY: Young’s modulus. NE: did not evaluate the variable in the study.

Table 1.2. Studies evaluating the morphological properties of the Achilles tendon and plantar flexors muscles of healthy individuals.

	Magnusson et al., 2001	**Duclay et al., 2009**	**Arya e Kulig, 2010.**	**Morrisey et al., 2011**	**Fouré et al., 2013**	**Geremia et al., 2015**	**Gomes et al., 2016**	**Geremia et al., 2018**
Morphological Properties								
TL (cm)	24.9	NE	25.2±3.2	18.3±2.9	21.9±1.9	23.5±6.4	NE	21.8±2.5
CSA (mm²)	78.1	NE	56.3± 5.6	69.8±11.15	59.2±11.6	58.7±6.9	62.3±13.5	63.4±7.8
FL (cm)	NE	2.1±0.9 (GM)	NE	NE	9.1±3.1 (GL) 5.8±1.1 (GM) 3.6± (SO)	NE	NE	NE
PA (º)	NE	NE	NE	NE	12.3±2.4 (GL) 23.4±3.0 (GM) 25.1±7.7 (SO)	NE	NE	NE
MT (cm)	NE	NE	NE	NE	NE	NE	5.8±0.5	NE

TL: Tendon length. CSA: cross-sectional area. FL: fascicle length. PA: pennation angle. MT: muscle thickness. GM: gastrocnemius medialis. GL: gastrocnemius lateralis. SO: soleus.

From the mechanical properties, the variable that presented a greater variation in the values magnitude between the studies was the modulus of elasticity or Young's Modulus, which reflects the tendon material properties. The reason for these differences is not entirely clear, but may be related to the subjects physical condition in each study who may have been sedentary or physically active. The level of physical activity may interfere with the modulus of elasticity in healthy subjects, and should be higher in those subjects who present greater daily mechanical overload on the Achilles tendon.

1.9 Psychometric Properties

Clinical practice involves measuring quantities for a variety of purposes, such as aiding diagnosis, predicting future patient outcomes, and serving as end points in studies or randomized trials. Measurements are almost always prone to various sorts of errors, which cause the measured value to differ from the true value. (BARLETT and FROST 2008).

The importance of measurement error depends upon the context in which the measurements in question are to be used (BARLETT and FROST 2008). For example, a certain degree of measurement error may be acceptable if measurements are to be used as an outcome in a comparative study such as a clinical trial.

Regardless of the device or protocol used for the functional evaluation, it is essential that the methodology used is reliable and that the results are not influenced by the rater or by the evaluation day and time. In other words, it is important that the variables being measured are not affected by a number of intervening factors, since they would alter the accuracy of the measurements and determine a great variability in the results (KOTTNER et al., 2011). This variability, in turn, would make it difficult to determine the psychometric properties of the obtained measurements, as well as to compare the results between studies. Accordingly, the outcome variables should have the characteristics of a good intra- and inter-rater reliability (OLIVEIRA et al., 2012).

Reliability refers to the degree of agreement between the results of measurements of the same magnitude when individual measurements are made by varying conditions such as: measurement method, observer, measuring instrument, location, and time conditions (BARLETT and FROST 2008). The term reliability is associated with the concept of precision. However, reliability is not defined by itself, and lacks complementarity for a clearer definition. Thus, some authors suggest certain terms to specify the reliability condition.

The inter-rater reliability is the degree of reproducibility in successive instants, varying the observer, but keeping the other conditions constant. The intra-rater reliability is the degree of measurements reliability, varying the time between the evaluations (with interval of at least one day), but keeping the other conditions constant (without varying rater) (OLIVEIRA et al., 2012, VIM, 2012). The inter-analyst reliability uses images regardless of who collected them, comparing the analysis done by different analyzers (SARWAL et al., 2015).

To determine intra- and inter-rater reliability, common metrics to quantify reliability are intra-class correlation coefficients (ICC), standard error of measurements (SEM) and minimal detectable change (MDC) (WEIR, 2005).

1.10 Intra- and inter-rater reliability of the triceps surae morphological and mechanical properties

To date, no studies have been found in the literature that evaluated the intra- and inter-rater reliability of the triceps surae mechanical and morphological properties. Some studies evaluated the variables separately. Konig et al. (2014) evaluated the medial gastrocnemius muscle architecture intra- and inter-rater reliability. Fifteen physically active individuals were recruited and high ICC values and low SEM values were identified (Table 1.3). Brouwer et al. (2018) evaluated the total Achilles tendon length reliability by comparing the US method and MRI in 20 individuals. The US method obtained higher values of intra and inter-rater ICC, with low values of SEM and MDC (Table 1.3). Bohm et al. (2016) obtained high ICC values for the Achilles tendon CSA intra- and inter-rater reliability. The area was measured by defining 3 points (proximal, medial and distal) from the total tendon length. More recently, Barfod et al. (2018) evaluated the free Achilles tendon intra- and inter-rater reliability through US. High ICC values and low values of SEM and MDC (Table 1.3) were presented for this measurement.

No studies were found that evaluated the Achilles tendon mechanical properties intra- and inter-rater reliability. A single study by Yamamoto et al. (2016) evaluated intra-rater repeatability of the Achilles tendon strain measurements. One hundred tendons of fifty subjects were used and a minimum detectable change between the raters of 0.03 ± 0.02 was found (mean and standard deviation of raters 1 and 2, respectively, 0.36 ± 0.14 and 0.39 ± 0.12). In the Achilles tendon strain rate, obtaining an intra-rater ICC (ICC 1, 3) of 0.93 (0.89-0.96) and 0.87 (0.80-0.92) between the measurements obtained.

Table 1.3. Studies evaluating the morphological properties of the Achilles tendon and plantar flexors muscles of healthy individuals.

	Intra-rater ICC	**Inter-rater ICC**	**SEM**	**MDC**
Morphological properties				
Konig et al (2014)	NE	0.82 (MT), 0.80 (PA) 0.77 (FL)	0.1 cm (MT), 1° (PA), 0.4 cm (FL)	NE
Brower et al., (2018)	0.96	0.96	0.5cm	1.4 cm
Bohm et al., (2016)	0.986	0.948	NE	NE
Barfod et al., (2018)	0.94	0.96	0.5cm	0.13cm

ICC: intra-class correlation coefficients, SEM: standard error of measurements, MDC: minimal detectable change.

It is important to observe that the main studies found in the literature are related to the evaluation and comparison of these properties between healthy individuals and Achilles tendon rupture patients. However, there is still a lack of evidence regarding the psychometric variables related to the evaluation of the triceps surae mechanical and morphological properties. The vast majority of the studies found did not have a strong methodological rigor, had three evaluators with a relatively low sample number, and used only the ICC value as a reference to say whether the measurement was reliable or not.

Thus, the adoption of a systematic and concise methodology for triceps sural assessment is necessary to improve the strategies for injury prevention and evaluation of the recovery process in rehabilitation programs (BRISSON et al., 2013). In addition, the reliability evaluation of the plantar flexors and the Achilles tendon in healthy young individuals could benefit the less experienced evaluators. Thus, by conducting such study, it will be possible to measure the instrument psychometric level and establish a strong methodology for the evaluation of tendinopathy patients and/or Achilles tendon rupture patients.

1.11 REFERENCES

ABELLANEDA, S., GUISSARD, N., DUCHATEAU, J. The relative lengthening of the myotendinous structures in the medial gastrocnemius during passive stretching differs among individuals. **Journal of Applied Physiology**, v.106, n.12, p.169-177, 2009.

ALVARES, J.B.A.R, RODRIGUES, R., FRANKE, R.A. et al. Inter-machine Reliability of the Biodex and Cybex Isokinetic Dynamometers for Knee Flexor-Extensor Isometric, Concentric and Eccentric Tests. **Physical Therapy in Sport**, v.16, n.1, p.59-65, 2015.

ARYA, S.; KULIG, K. Tendinopathy Alters Mechanical and Material Properties of the Achilles Tendon. **Journal of Applied Physiology,** v.108, n.3, p.670-5, 2010.

ARAMPATZIS, A.; KARAMANIDIS, K.; ALBRACHT, K. Adaptational Responses of the Human Achilles Tendon by Modulation of the Applied Cyclic Strain Magnitude. **The Journal of Experimental Biology,** v.210, n.15, p.2743-53, 2007.

BALTZPOULOS, V., BRODIE, D.A. Isokinetic Dynamometry. Applications and Limitations. **Sports Medicine**, v.8, n.2, p.101-116, 1989.

BARFOD, K.W. Achilles tendon rupture; assessment of nonoperative treatment. **Danish Medical Journal**, v.61, n.4, p.48-59, 2014.

BARFOD K.W., RIECKE, A.F, BOESEN, A. et al. Validation of a novel ultrasound measurement of Achilles tendon length. **Knee Surgery, Sports Traumatology, Arthroscopy**, v.23, n.11, p.3398-3406, 2015.

BARFOD, K.W.; RIECKE, A.F.; BOESEN, A. et al. Validity and reliability of an ultrasound measurement of the free length of the Achilles tendon. **Danish Medical Journal,** v.65, n.3, p.65-70, 2018.

BARONI, B.M., GEREMIA, J.M., RODRIGUES, R. et al. Muscle architecture adaptations to knee extensor eccentric training: rectus femoris vs. vastus lateralis. **Muscle and Nerve**, v.48, n.4, p.498-506, 2013.

BARTLETT, J.W., FROST, C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. **Ultrasound Obstetrics Gynecology,** v.31, n.4, p.466-75, 2008.

BJUR, D., ALFREDSON, H., FORSGREN, S. Presence of the neuropeptide Y1 receptor in tenocytes and blood vessel walls in the human Achilles tendon. **British Journal of Sports Medicine**, v.43, n.14, p.1136-1142, 2009.

BLAZEVICH, A.J., Effects of physical training and detraining, immobilisation, growth and aging on human fascicle geometry. **Sports Medicine**, v.36, n.12, p.1003-1017, 2006.

BRAMBLE, D.M; LIEBERMAN, D.E. Endurance running and the evolution of Homo. **Nature**, v.32, n.7015, p.345-352, 2004.

BRISSON, N., LAMONTAGNE, M., KENNEDY, M. J. ET ALThe effects of cam femoroacetabular impingement corrective surgery on lower-extremity gait biomechanics. **Gait and Posture**, v.37, n.2, p.258-263, 2013.

BROUWER, F.E., MYHRVOLD, S.B., BENTH, J.S et al. Ultrasound measurements of Achilles tendon length using skin markings are more reliable than extended-field-of-view imaging. **Knee Surgery, Sports Traumatology, Arthroscopy,** v.26, n.7, p.2088-2094, 2018.

BRUSHOJ, C., HENRIKSEN, B.M, ALBRECHT-BESTE, E. et al. Reproducibility of ultrasound and magnetic resonance imaging measurements of tendon size. **Acta Radiologica**, v. 47, n.9, p.954-959, 2006.

BOHM S., MERSMANN, F, ARAMPATZIS, A. Human tendon adaptation in response to mechanical loading: a systematic review and meta-analysis of exercise intervention studies on healthy adults. **Sports Medicine – Open***,* v.1, n.7, p.80-92, 2015.

BOHM, S., MERSMANN, F., SCHROLL, A. et al. Insufficient accuracy of the ultrasound-based determination of Achilles tendon cross-sectional area. **Journal of Biomechanics,** v.49, n.13, p.2932-2937, 2016.

DORAL, M.N., ALAM, M., BOZKURT, M. et al. Functional anatomy of the Achilles tendon. **Knee Surgery, Sports Traumatology, Arthroscopy**, v.18, n.5, p.638-643, 2010.

DUCLAY, J.; MARTIN, A.; DUCLAY, A. et al. Behavior of Fascicles and the Myotendinous Junction of Human Medial Gastrocnemius Following Eccentric Strength Training**. Muscle and Nerve**, v.39, n.6, p.819-27, 2009.

EKIZOS, A., PAPATZIKA, F., CHARCHARIS, G. et al (2013). Ultrasound does not provide reliable results for the measurement of the patellar tendon cross sectional area. **Journal of Electromyography and Kinesiology**, v.23, n.6, p.1278-1282.

FINNI, T. Structural and functional features of human muscle-tendon unit. Scandinavian **Journal of Medicine & Science in Sport,** v.16, n.3, p.147-158, 2006.

FUKUNAGA, T., KAWAKAMI, Y., KUNO, S., FUNATO, K. et al. Muscle architecture and function in humans. **Journal of Biomechanics**, v.30, n.5, p.457-463, 1999.

FOURÉ, A., NORDEZ, A., MCNAIE, P., CORNU, C. Effects of plyometric training on both active and passive parts of the plantarflexors series elastic component stiffness of muscle-tendon complex. **European Journal of Applied Physiology,** v.111, n. 15, p.539-548, 2011.

FRAÇÃO, V. B., VAZ, M. A. Influência da adaptação funcional na capacidade de produção de força no músculo esquelético. **Revista Perfil**, v.4, n.4, p.103-109, 2000.

GEREMIA, J.M.; BOBBERT, M.F.; CASA NOVA, M. et al. The structural and mechanical properties of the Achilles tendon 2 years after surgical repair. **Clinical Biomechanics**, v.30, n.5, p. 485-92, 2015.

GEREMIA, J.M., BARONI, B.M., BOBBERT, M.F. et al. Effects of high loading by eccentric triceps surae training on Achilles tendon properties in humans. **European Journal of Applied Physiology**, v.118, n.8, p.1725-1736, 2018.

GILES, L.S., WEBSTER, K.E., MCCLELLAND, J.A., COOL, J. Does quadriceps atrophy exist in individuals with patellofemoral pain? A systematic literature review with meta-analysis. **Orthopaedics Sports and Physical Therapy,** v.43, n.11, p.766-776, 2013.

GOMES, A.R.S, CAMPOS, T.F, BECKENKAMP, R. Effects of isokinetic eccentric training on the human Achilles tendon. **Journal of Exercise Physiology**, v.19, n.2, p.46-54, 2016.

HERZOG W., GUIMARAES, A.C., ANTON, M.G et al. Moment-length relations of rectus femoris muscles of speed skaters/cyclists and runners. **Medicine and Science in Sports and Exercise**, v.23, n.3, p.1289-1296, 1991.

IMPELLIZZEERI, F.M., BIZZINI, M., RAMPININI, E. et al. Reliability of isokinetic strength imbalance ratios measured using the Cybex NORM dynamometer. **Clinical Physiology and Functional Imaging**, v. 28, n.7, p.113-119, 2008.

KARAMANIDIS, K.; ARAMPATZIS, A. Mechanical and morphological properties of human quadriceps femoris and triceps surae muscle-tendon unit in relation to aging and running. **Journal of Biomechanics**, v.39, n.3, p.406-417, 2005.

KOMI, P. **Strength and Power in Sport**. Vol 3. Encyclopaedia of Sports Medicine, 1992.

KANNUS, P., JOZSA, L., NATRI,J. et al. Effects of training, immobilization and remobilization on tendons. **Scandinavian Journal of Medicine and Science in Sports**, v.7, n2, p.67-71, 1997.

KOTTNER, J., AUDIGÉ, L., BROSON, S., DENNER, A., GAJEWSKI, B.J., HRÓBJARTSSON, A., ROBERTS, C., SHOUKRI, M., STREINEI, D.L. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. **Journal of Clinical Epidemiology,** v.64, n.1, p.96-106, 2011.

KONIG, N., CASSEL, M., INTZIEGIANNI, K., MAYER, F. Inter-rater reliability and measurement error of sonographic muscle architecture assessments. **Journal of Ultrasound in Medicine**, v.33, n.5, p.769-777, 2014.

LIEBER, R. L., FRIDEN, J. Functional and clinical significance of skeletal muscle architecture. **Muscle & Nerve**, v.23, n.11, p.1647-1666, 2000.

MADEMLI, L.; ARAMPATZIS, A.; MOREY-KLAPSING, G. et al. Effect of Ankle Joint Position and Electrode Placement on the Estimation of the Antagonistic Moment During Maximal Plantarflexion. **Journal of Electromyography and Kinesiology,** v.14, n.5, p.591-7, 2005.

MAFFULLI, N., ALMEKINDERS, LC. **The Achilles Tendon**. First Edition, *Springer*, 2007.

MAGNUSSON, S. P.; AAGAARD, P.; DYHRE-POULSEN, P. et al. Load-Displacement Properties of the Human Triceps Surae Aponeurosis in Vivo. **The Journal of Physiology,** v.531, n.1, p.277-88, 2001.

MORRISEY, D., ROSKILLY, A. A., TWYCROSS-LEWIS, R., et al. The effect of eccentric and concentric calf muscle training on Achilles tendon stiffness. **Clinical Rehabilitation**, v.25, n.3, p.238-247, 2011.

NARICI, M. Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. **Journal of Electromyography and Kinesiology**, v.9, n.2, p.97-103, 1999.

NAKAMURA, M., IKZOE, T., TAKENO, Y. et al. Acute and prolonged effect of static stretching on the passive stiffness of the human gastrocnemius muscle tendon unit in vivo. **Journal of Orthopaedic Research**, v.29, n.11, p.1759-1763, 2011.

NIGG, B. M.; HERZOG, W. **Biomechanics of the Musculo-Skeletal System.** Toronto: John Wiley & Sons, 1999.

OLIVEIRA, T. S., CANDOTTI, C. T., LA TORRE, M. et al. Validity and Reproducibility of the Measurements Obtained Using the Flexicurve Instrument to Evaluate the Angles of Thoracic and Lumbar Curvatures of the Spine in the Sagittal Plane. **Rehabilitation Research and Practice,** v.6, n.2, p.1-9, 2012.

PIERRE-JEROME, C., MONCAYO, V., TERK, M.R. MRI of the Achilles tendon: a comprehensive review of the anatomy, biomechanics, and imaging of overuse tendinopathies. **Acta Radiologica**, v.51, n.4, p.438-453, 2010.

REES, J.D., LICHTWARK,G.A., WOLMAN, R.L. et al. The mechanism for efficacy of eccentric loading in Achilles tendon injury; an in vivo study in humans. **Rheumathology,** v.47, n.10, p.1493-1497, 2008.

REEVES, N.D, MAGANARIS, C.N, LONGO, S., NARICI, M.V. Differential adaptations to eccentric versus conventional resistance training in older humans. **Experimental Physiology**, v.94, n.7, p.825-833, 2009.

SARWAL, A., PARRT, S,M., BERRY, M.J., HSU, F.C., LEWIS, M.T., JUSTUS, N.W., MORRIS, P.E, DENEHY, L., BERNEY, S., DHAR, S., CARTWRIGHT, M.S. Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. **Journal of Ultrasound in Medicine,** v.34, n.7, p.1191-200, 2015.

SEYNNES, O. R., ERSKINE, R. M., Maganaris, C. N. et al. Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. **Journal of Applied Physiology**, v.107, n.2, p.523-530, 2009.

SILVER, F.H., FREEMAN, J.W., SEEHRA, G.P. Collagen self-assembly and the development of tendon mechanical properties. **Journal of Biomechanics**, v.36, n.10, p.1529-53, 2003.

STENROTH, L., PELTONEN, J., CRONIN, N. Age-related differences in Achilles Tendon properties and triceps surae muscle architecture in vivo. Journal Applied **Physiology,** v.113, n.23,p.1537-1544, 2012.

URLANDO, A, HAWKINS, D. Achilles tendon adaptation during strength training in young adults. **Medicine and Science in Sports and Exercise**, v.39, p.1147-1152, 2007.

VIM. **International Vocabulary of Metrology** – Basic and general concepts and associated terms (3rd ed.). Joint Committee for Guides in Metrology: 2012.

WANG, C.C., CHEN, P.Y., WANG, T.M, WANG, C.L. Ultrasound-guided minimally invasive surgery for Achilles tendon rupture: preliminary results. **Foot & Ankle International**, v.33, n.5, p.582-590, 2012.

WANG, J.H-C. Mechanobiology of tendon**. Journal of Biomechanics**, v.39, n.9, p.1563-82, 2006.

WARD, S.R., SARVER, J.J., ENG, C.M., KWAN, A., WURGLER-HAURI, C.C., PERRY, S.M., WILLIAMS, G.R., SOSLOWSKY, L.J., LIEBER, R.L. Plasticity of muscle architecture after supraspinatus tears**. The Journal of Orthopaedic and Sports Physical Therapy**, v.40, n.11, p.729-735, 2009.

WEIR, J.P. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. **Journal of Strength and Conditioning Research**, v.19, n.1, p.231-40, 2005.

WHITTAKER, J. Current perspectives: the clinical application of ultrasound imaging by physical therapists. **Journal of Manual & Manipulative Therapy**, v.14, n.2, p.73-75, 2006.

YAMAMOTO, Y.; YAMAGUCHI, S.; SASHO, T. et al. Quantitative Ultrasound Elastography with an Acoustic Coupler for Achilles Tendon Elasticity: Measurement Repeatability and Normative Values. **Journal of Ultrasound in Medicine,** v.35, n.1, p.159-166, 2016.

CHAPTER 2

INTRA-RATER, INTER-RATER AND INTER-ANALYST RELIABILITY OF ULTRASOUND-DERIVED TRICEPS SURAE MUSCLE ARCHITECTURE

Francesca Chaida Sonda, Emmanuel Souza da Rocha, Eliane Celina Guadagnin, Rodrigo Rabello da Silva, Jeam Marcel Geremia, Viviane Bortoluzzi Frasson, Marco Aurélio Vaz

2.1 INTRODUCTION

In the last two decades, biomechanics started the ultrasonic study of in vivo muscle architecture (BAPTISTA and VAZ, 2009). Muscle ultrasound studies were validated by anatomical measurements in human cadavers (KAWAKAMI et al., 1993; NARICI et al., 1996). Ultrasonography (US) allowed the assessment of muscle plasticity induced by both increased use (physical training) and reduced use (immobilization and microgravity) and aging (KAWAKAMI et al.,1995; KAWAKAMI et al., 2000; AAGARD et al., 2001; BIOLO et al., 2003).

In clinical practice, muscle structures' US is one of the most frequent and cost-effective methods used by physicians (WALKER et al., 2004). It allows the diagnosis of structural lesions (WALKER et al., 2004), as well as the identification of injury severity in tendon and muscle ruptures (MEYER et al., 2011; KIM et al., 2012). Moreover, muscle US examinations can help to prevent injuries (KONIG et al., 2014). Therefore, muscle architecture US examinations allow a more comprehensive understanding of muscle-related disorders as well as injury mechanisms, and, as a consequence, it might have an impact on the clinical decision-making process (WARD et al., 2010; KIM et al., 2012).

US is a non-invasive technique that has some advantages: it does not emit ionizing radiation, it is cheaper and it allows real-time static and dynamic evaluations of muscle architecture (GILES et al., 2015). However, it is an evaluator-dependent technique (MENDIS et al., 2010), which suggests that a large volume of practice is necessary before it can be used in clinical practice. During ultrasound measurements, the probe location and orientation are crucial for the accuracy of the muscle architecture parameters (AGELOUSSIS et al., 2010). Both are quite difficult to be reproduced and their variation may induce random errors in repetitive measurements (GIANNAKOU et al., 2011). Thus, the degree of reliability of its measurements depends on a strict protocol and knowledge of the analyzed structures by the examiner (KOTTNER et al., 2011).

Regardless of the device or protocol used in the functional assessment, it is essential for the methodology to be reliable so that the results are not influenced by the examiner or by the chronobiology (effect of the time). In other words, it is important that the measured variables are not affected by a number of intervening factors, since they would alter the measurements precision, which could lead to an excessive variability in the results (TORRESAN, 2016). This variability could hinder the determination of the measurements' psychometric properties, as well as the between-studies results comparison (OLIVEIRA et al., 2012).

Although the terminology is not constant between authors, and there are different kinds of reliability, three are commonly found in the literature. The intra-rater reliability compares the data collected by the same rater in different moments (WALTER et al., 1998, VIM, 2012). The inter-rater reliability compares the data collected by different raters in the same day (SEDREZ et al., 2018). The inter-analyst reliability uses images regardless of who collected them, comparing the analysis done by different analyzers (SARWAL et al., 2015).

Previous studies evaluated the intra-rater reliability of US measures and presented, in general, good to excellent results for knee extensors (BARONI et al., 2013), and gastrocnemius medialis (NARICI, 1996; AGGELOUSSIS et al., 2010; GIAAAKOU et al., 2011), considering repeated measures with the individuals at rest and performing dynamic tasks. Konig et al. (2014) evaluated gastrocnemius medialis muscle architecture inter-rater reliability in healthy subjects. They found a high inter-rater Intra-Class Correlation Coefficient (ICC) for fascicle length, pennation angle and muscle thickness (r: 0.82, 0.90, and 0.77, respectively). As for the inter-analyst reliability, to the best of our knowledge, only one study evaluated quadriceps and diaphragm muscles in critical care patients (SARWAL et al., 2015). They found high ICC values (r: 0.84 to 0.99). We were unable to find studies that measured intra-rater, inter-rater and inter-analyst reproducibility of the plantar flexor muscles in healthy young individuals.

Considering the lack of evidence about the quantification of psychometric variables of plantar flexor muscles US assessment, research concerning these aspects is still needed. Moreover, this information could benefit less experienced evaluators and serve as normative data for future comparison with other populations such as individuals with clinical, structural and/or functional alterations in the ankle joint. Furthermore, the adoption of a systematic and concise methodology for assessing the plantar flexor muscles' structure is necessary to improve the evaluations quality, allowing a better understanding of the structure, helping the rehabilitation process and permitting the development of strategies for injuries prevention.

2.2 PURPOSE

The main goal of the present study was to evaluate the intra-rater (measurements performed on different days by the same rater), inter-rater (measurements performed on the same day by three independent raters) and inter-analyst (images analyzed digitally by two different analysts) reliability of US triceps surae muscle architecture measurements. To accomplish this, we evaluated: (1) gastrocnemius medialis fascicle length (2) gastrocnemius lateralis fascicle length, (3) gastrocnemius medialis fascicle pennation angle (4) gastrocnemius lateralis fascicle pennation angle (5) gastrocnemius medialis muscle thickness (6) gastrocnemius lateralis muscle thickness (7) soleus muscle thickness. The hypothesis raised in the present study was that all measures would have good reproducibility as an adequate training of the raters' team was carried out before the tests.

2.3 METHODS

The evaluations took place at the Neuromuscular Plasticity Department of the Exercise Research Laboratory (LAPEX, Porto Alegre, Brazil) of the Physical Education, Physiotherapy and Dance Faculty (ESEFID - UFRGS). This study used a methodology approved by the University's Research Ethics Committee (CAEE nº 79420817.6.0000.5347). Participants read and signed the informed consent form after they had all questions about the tests to be performed answered by the responsible researcher.

2.3.1 Sample

Sample size calculation was in accordance with the current literature that investigated the gastrocnemius medialis US reliability (AGELOUSSIS et al., 2010, GIANNAKOU et al., 2011). Sample size was calculated with the G*Power software (Version 3.1, Kiel University, Germany). We assumed the ICC null hypothesis value to be 0.40 (e.g. on the basis that any value lower than 0.40 might be considered clinically “unacceptable”); 80% of power; two replicated measurements (one for each evaluator or twice by the same evaluator); and a significance level of 95% to detect an ICC value of 0.70 based on previous literature (PORTNEY et al., 2009). A sample of 15 individuals was defined as the minimum number of subjects. However, an additional fifty percent of drop-out rate was included to consider those respondent(s) who would fail to attend the follow-up session (i.e. re-test). Furthermore, because we wanted to evaluate the measurements reliability in both sexes, 15 subjects from each sex were recruited totalizing 30 individuals.

2.3.2 Participants

Healthy and physically active university male (15) and female (15) subjects between 18-35 years old (25.6±4.2 years; height = 1.70±0.09 m; weight = 69.21±7.83 kg; all with right dominance) volunteered to participate in the study after disclosure of the project on the University's campus and by social networks. Participants were included if they: (1) had no lower or upper limb musculoskeletal injuries; (3) had no contraindication to perform maximal strength tests (e.g. cardiovascular, respiratory, neurological and musculoskeletal diseases); (4) had no difficulty to understand and/or execute the protocol tests; (5) did not miss any of the performed tests.

2.3.3 Experimental design

This study was characterized as a transversal correlational study, aimed at verifying intra-rater, inter-rater, and inter-analyst reliability of plantar flexors' (gastrocnemius medialis, gastrocnemius lateralis and soleus) muscle architecture.

After reading the consent form, participants were submitted to two assessment days separated by seven days. At the first session (1), the assessment was performed by evaluator 1, while at the second session (2) the same assessment was performed by evaluators 1, 2 and 3, successively (Figure 2.1).

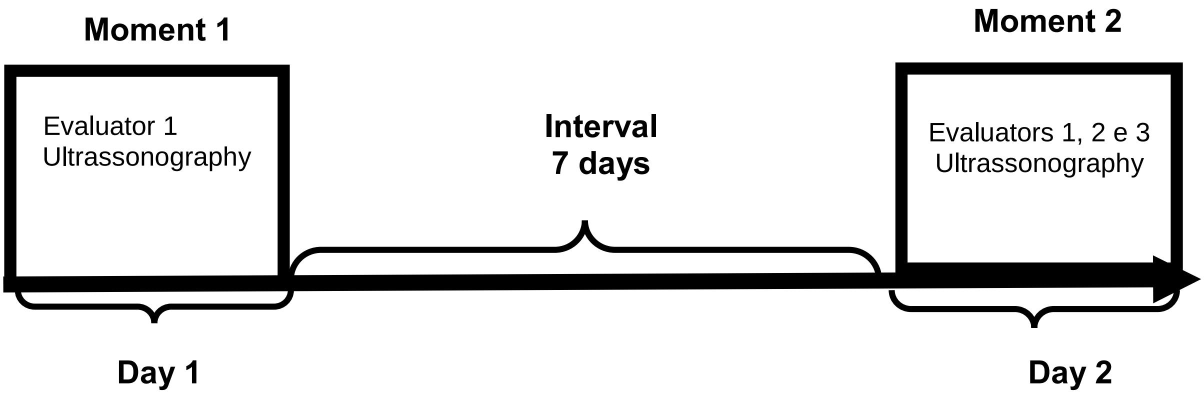


Figure 2.1. Experimental Design including intra and inter-rater reliability.

2.3.4 Procedures

Three evaluators with six months to two years of musculoskeletal US experience (evaluator 1: two years; evaluator 2: one year months; evaluator 3 = six months) conducted all tests. They used the same methodology for all evaluations, and the evaluators order was randomly determined through the website www.random.org.

An additional researcher assisted the evaluators with the lab organization and the participant's positioning in each test. The three evaluators did not have contact during the procedures and were not in the lab during the data collection of the other two evaluators. The plantar flexors muscle architecture of each participant was assessed through US, and each session lasted approximately 20 minutes.

2.3.5 Evaluation protocol

At the first evaluation, height and body mass were measured according to the International Society for Advancement of Kineanthropometry (Marfell-Jones et al. 2006). Participants rested for 10 minutes before the US tests and were instructed not to engage in any vigorous physical activity for the previous 48 hours for each evaluation (BARONI et al., 2013). Muscle architecture was evaluated with a B-mode US system (LOGIQ P6, GE Healthcare, Waukesha, Washington, United States of America) and a matrix linear array transducer (50mm, 15 MHz - GE Healthcare, Waukesha, Washington, United States of America). The frequency was set to 9 MHz, the depth to 6 cm, and the focus was dynamically adjusted by the US operator. Great care was taken to determine the specific sites where the images were collected. Anatomical and reference points were marked on the skin. The reference-points were marked at the midpoint popliteal fold and at the lateral malleolus center. After each evaluation, the marks on the skin were removed in order to secure blinding between evaluators.

The images were obtained with the subjects in the ventral decubitus, with the knees fully extended (0°) and the ankle in neutral position (heel line at a 90° angle with respect to the longitudinal axis of the leg, 0° of plantarflexion). A custom system was used to secure the ankle in the neutral position (Figure 2.2). The ankle joint position was controlled using a digital goniometer (DIGIMESS, United States of America) (Figure 2.3).

Figure 2.2. Ankle in neutral position for morphological properties measurements.

Figure 2.3. Digital goniometer used to control the position of the ankle joint.

2.3.6 Evaluation of muscle architecture parameters

The assessed muscle architecture parameters were fascicle length, pennation angle and muscle thickness (NARICI, 1999). The US probe was positioned longitudinally the muscle fibers, at 30% proximal of the distance between the popliteal fold and the lateral malleolus center for the medial and lateral gastrocnemius, and at 50% for the soleus muscle (KAWAKAMI *et al*., 1998) (Figure 2.4).

Figure 2.4. Positioning of the probe for the medial gastrocnemius, soleus and lateral gastrocnemius muscles respectively.

The probe was oriented parallel to the muscular fascicles, and perpendicular to the skin over plantar flexors muscles. Thus, the probe orientation relative to the longitudinal axis of the calf was different between subjects due to their individual anatomical characteristics. Probe alignment was considered appropriate when several fascicles could be easily delineated without interruption across the image (BARONI *et al*., 2013). Three images were obtained from each muscle.

2.3.7 Data analysis

Muscle architecture was analyzed by two analysts with extensive experience. Blinding was achieved by a random-number code that was assigned to each image before testing and saved in a "master file". This procedure was carried out by a research assistant.

During the tests, images were saved according to a random-number code to ensure that the image analysts were unaware of the identification of the participants' images. US images were digitized and analyzed with *ImageJ* version 1.46k23 software (National Institutes of Health, Bethesda, Maryland). All three images from each muscle were analyzed, resulting in a total of 270 images (3 [muscles] x 3 [evaluators] x 30 [participants]). The outcomes mean value was considered for statistical analysis.

To calculate the muscle thickness, five equidistant measurements of the distance between the superficial and deep aponeuroses from the gastrocnemius medialis and lateralis muscles were performed (BARONI et al., 2013). For the soleus muscle, only muscle thickness was analyzed due to the difficulty in finding the fascicle length in the image.

For the fascicle length and pennation angle outcomes, the best fascicle in each image was used for pennation angle and fascicle length analysis (Figure 2.5). When the end of the fascicle extended beyond the acquired ultrasound image, fascicle length was estimated by trigonometry while assuming a linear projection of the fascicles (ARAMPATZIS et al., 2005; ABELLANEDA et al., 2009). The error due to the linear extrapolation has been estimated to be in the range of 2-7% (FINNI et al., 2003). Pennation angle was calculated as the angle between the best fascicle and the deep aponeurosis (KUBO et al., 2003a; BARONI et al., 2013; GEREMIA and VAZ, 2016). Following the image analysis, the measured results were assigned to the participants' random-number codes. The mean value of the three images from each muscle was considered for statistical analysis.

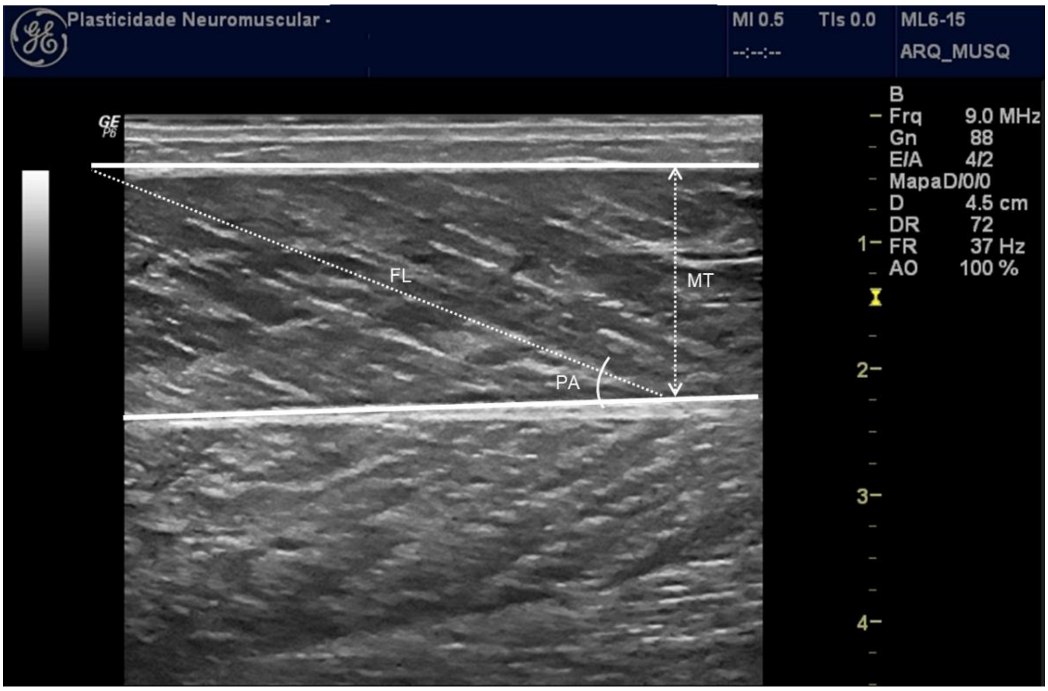


Figure 2.5. Gastrocnemius medialis muscle architecture measurement. MT = muscle thickness; PA = pennation angle; FL = fascicle length.

2.3.8 Statistical Analysis

The statistical analysis was performed with the software SPSS 22.0. Initially, mean and standard deviation (SD) values were used in the descriptive analysis. The data normality was verified using the Shapiro-Wilk test. Values obtained by the same rater in different days were used to obtain the intra-rater reliability. Values obtained by different raters in the same day were used to obtain the inter-rater reliability. Values obtained by different analysts were used to obtain the inter-analyst reliability. To verify the intra-, inter-rater and inter-analyst reliability, the ICC, the Standard Error of the Measurement (SEM), the Minimum Detectable Change (MDC), and the Coefficient of Variation (CV) were calculated. The ICC values were classified according to the literature (PORTNEY and WATKINS, 2009; SCHWENK *et al*., 2012) as weak (r<0.40), moderate (0.40 > r < 0.75), and excellent (r>0.75). The Standard Error of the Measurement (SEM) was estimated using the following equation: $SEM=SD*\sqrt{1-ICC}$ where SD is the standard deviation of the measurements. The Minimum Detectable Change (MDC) was estimated based on a 95% confidence interval, where *MDC=1.96*SEM*. The coefficient of variation was calculated using the equation: CV=SD/X*100, where X is the mean value. A significance level of 5% was adopted for all analyzes.

2.4 RESULTS

Mean±SD values for all outcomes are presented in Table 2.1. The mean ± SD values, ICC, confidence interval (CI), (SEM), (MDC), and (CV) for GM_{FL}, GL_{FL}, GM_{PA}, GL_{PA}, GM_{MT}, GL_{MT} and SO_{MT} for the raters and analysts are presented in tables 2 to 8.

All intra-rater comparisons showed a high ICC value (r > 0.75). The SEM and MDC values for this comparison were low. In relation to the inter-analyst comparisons, all ICCs were high (r >0.75), except for the GM_{FL} (r: 0.845), GL_{FL} (r: 0.766), and GL_{PA}, (r: 0.894). Correlation values were significant for all comparisons (p<0.001).

In the inter-rater comparisons (Tables 2 to 8), the parameters GM_{FL}, GL_{FL}, GM_{MT}, GL_{MT}, SO_{MT} showed high ICCs (r > 0.75). The GM_{PA} and GL_{PA} also presented high ICCs (r: 0.766 to 0.896). The SEM and MDC values were low for GM_{FL} and GL_{FL} (0.13cm to 0.57cm), GM_{PA} and GL_{PA} (0.41º to 2.82º), and GM_{MT}, GL_{MT} and SO_{MT} (0.03cm to 0.19cm). For the between-raters comparisons, GM_{FL}, GL_{FL}, GM_{PA}, GL_{PA}, GM_{MT} and GL_{MT} showed high ICCs between raters 1 and 2 (r: 0.948; 0.953; 0.865; 0.817; 0.974; 0.953). SO_{MT} showed high ICCs between raters 2 and 3 (r: 0.968). The SEM and MDC values were low for GM_{FL} (0.15cm to 0.29cm), GL_{FL} (0.15cm to 0.29cm), GM_{PA} (0.97º to 1.90º), GL_{PA} (0.96º to 1.87º), GM_{MT} (0.05cm to 0.10cm), and GL_{MT} (0.04cm to 0.09cm), between raters 1 and 2. The SEM and MDC values were low for SO_{MT} between raters 2 and 3 (0.05cm to 0.09cm). For inter-analyst comparisons of GM_{FL}, GL_{FL}, GM_{PA}, GL_{PA}, GM_{MT}, GL_{MT}, and SO_{MT} measurements, ICCs were high (r > 0.80), while SEM and MDC values were low in relation to the mean values. Correlation values were significant for all comparisons (p<0.001).

Table 2.1: Mean and standard deviation values of ultrasound measurements acquired in two different days by the same evaluator and in the same day by three different evaluators.

	Intra-Rater		**Inter-Rater**	
Ultrasound measurements	**1st Day** (Mean ± SD)	**2nd Day (Rater 1)** (Mean ± SD)	**Rater 2** (Mean ± SD)	**Rater 3** (Mean ± SD)
GM_{FL} (cm)	5.35 ± 0.61	5.33 ± 0.67	5.34 ± 0.64	5.23 ± 0.84
GL_{FL} (cm)	4.40 ± 0.87	4.42 ± 0.87	4.38 ± 0.80	4.38 ± 1.01
GM_{PA} (degrees)	22.30 ± 2.45	22.16 ± 2.41	22.88 ± 2.85	22.27 ± 2.58
GL_{PA} (degrees)	16.26 ± 2.43	16.12 ± 2.33	16.29 ± 2.17	16.74 ± 1.96
GM_{MT} (cm)	1.84 ± 0.27	1.83 ± 0.32	1.87 ± 0.30	1.83 ± 0.28
GL_{MT} (cm)	1.34 ± 0.21	1.33 ± 0.19	1.34 ± 0.21	1.37 ± 0.20
SO_{MT} (cm)	1.48 ± 0.23	1.48 ± 0.24	1.49 ± 0.25	1.47 ± 0.24

GM_{FL} = gastrocnemius medialis fascicle length; **GL_{FL}** = gastrocnemius lateralis fascicle length; **GM_{PA}** = gastrocnemius medialis pennation angle; **GL_{PA}** = gastrocnemius lateralis pennation angle; **GM_{MT}** = gastrocnemius medialis muscle thickness; **GL_{MT}** = gastrocnemius lateralis muscle thickness; and **SO_{MT}** = soleus muscle thickness.

Table 2.2 Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius medialis fascicle length ($GM_{FL.}$).

Gastrocnemius Medialis Fascicle Length							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	5.34 ± 0.64 cm	0.921	0.834-0.962	<0.001	0.18 cm	0.35 cm	11.91 %
Inter-analyst intra-rater	5.38 ± 0.74 cm	0.845	0.730-0.919	<0.001	0.29 cm	0.57 cm	13.74 %
Inter-rater	5.30 ± 0.72 cm	0.920	0.854-0.959	<0.001	0.20 cm	0.40 cm	13.53 %
Inter-rater (EV 1 *VS* EV 2)	5.33 ± 0.65 cm	0.948	0.892-0.976	<0.001	0.15 cm	0.29 cm	12.18 %
Inter-rater (EV 1 *VS* EV 3)	5.28 ± 0.76 cm	0.854	0.693-0.930	<0.001	0.29 cm	0.57 cm	14.34 %
Inter-rater (EV 2 *VS* EV 3)	5.29 ± 0.74 cm	0.865	0.716-0.936	<0.001	0.27 cm	0.54 cm	14.08 %
Inter-analyst inter-rater	5.32 ± 0.81 cm	0.897	0.827-0.945	<0.001	0.26 cm	0.51 cm	15.24 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation; EV: evaluator; ***VS****:* versus.

Table 2.3. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius lateralis fascicle length (GL_{FL}).

Gastrocnemius Lateralis Fascicle Length							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	4.41 ± 0.86 cm	0.978	0.953-0.989	<0.001	0.13 cm	0.25 cm	19.58 %
Inter-analyst intra-rater	5.10 ± 1.23 cm	0.766	0.591-0.878	<0.001	0.59 cm	1.17 cm	15.37 %
Inter-rater	4.39 ± 0.89 cm	0.949	0.906-0.974	<0.001	0.20 cm	0.39 cm	20.23 %
Inter-rater (EV 1 *VS* EV 2)	4.40 ± 0.83 cm	0.953	0.900-0.974	<0.001	0.18 cm	0.35 cm	18.82 %
Inter-rater (EV 1 *VS* EV 3)	4.40 ± 0.94 cm	0.923	0.838-0.963	<0.001	0.26 cm	0.51 cm	21.27 %
Inter-rater (EV 2 *VS* EV 3)	4.38 ± 0.91 cm	0.902	0.795-0.953	<0.001	0.28 cm	0.56 cm	20.68 %
Inter-analyst inter-rater	5.17 ± 1.32 cm	0.845	0.740-0.917	<0.001	0.52 cm	1.02 cm	25.53 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. **EV**: evaluator; **VS**: versus.

Table 2.4. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius medialis pennation angle (GM_{PA}).

Gastrocnemius Medialis Pennation Angle							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (º)**	**MDC (º)**	**CV (%)**
Intra-rater	22.13 ± 2.41°	0.916	0.824-0.960	<0.001	0.70°	1.37°	10.83 %
Inter-analyst intra-rater	21.52 ± 2.82°	0.927	0.873-0.962	<0.001	0.76°	1.49°	24.09 %
Inter-rater	22.44 ± 2.61°	0.896	0.809-0.947	<0.001	0.84°	1.65°	11.61 %
Inter-rater (EV 1 VS EV 2)	22.52 ± 2.64°	0.865	0.717-0.936	<0.001	0.97°	1.90°	11.71 %
Inter-rater (EV 1 VS EV 3)	22.22 ± 2.47°	0.847	0.679-0.927	<0.001	0.97°	1.90°	11.13 %
Inter-rater (EV 2 VS EV 3)	22.58 ± 2.71°	0.842	0.668-0.925	<0.001	1.08°	2.11°	12.00 %
Inter-analyst inter-rater	21.63 ± 3.12°	0.934	0.890-0.965	<0.001	0.84°	1.24°	14.42 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. EV: evaluator; ***VS***: versus.

Table 2.5. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius lateralis pennation angle (GL_{PA}).

Gastrocnemius Lateralis Pennation Angle							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (º)**	**MDC (º)**	**CV (%)**
Intra-rater	16.19 ± 2.36°	0.970	0.937-0.986	<0.001	0.41°	0.80°	14.59 %
Inter-analyst intra-rater	15.02 ± 2.56°	0.894	0.816-0.945	<0.001	0.83°	1.63°	19.75 %
Inter-rater	16.38 ± 3.71°	0.850	0.725-0.924	<0.001	1.44°	2.82°	22.64 %
Inter-rater (EV 1 *VS* EV 2)	15.27± 2.24°	0.817	0.615-0.913	<0.001	0.96°	1.87°	14.63 %
Inter-rater (EV 1 *VS* EV 3)	16.43± 2.16°	0.769	0.514-0.890	<0.001	1.04°	2.03°	13.12 %
Inter-rater (EV 2 *VS* EV 3)	16.51± 2.06°	0.783	0.544-0.897	<0.001	0.96°	1.89°	12.50 %
Inter-analyst inter-rater	14.91± 2.75°	0.856	0.759-0.923	<0.001	1.04°	2.05°	18.46 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. **EV**: evaluator; ***VS***: versus.

Table 2.6. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius medialis muscle thickness (GM_{MT}).

Gastrocnemius Medialis Muscle Thickness							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	1.83 ± 0.29 cm	0.967	0.931-0.984	<0.001	0.05 cm	0.10 cm	15.97 %
Inter-analyst intra-rater	1.79 ± 0.30 cm	0.979	0.963-0.989	<0.001	0.04 cm	0.09 cm	13.11 %
Inter-rater	1.84 ± 0.30 cm	0.970	0.946-0.985	<0.001	0.05 cm	0.10 cm	16.06 %
Inter-rater (EV 1 *VS* EV 2)	1.85 ± 0.31 cm	0.974	0.945-0.988	<0.001	0.05 cm	0.10 cm	16.53 %
Inter-rater (EV 1 *VS* EV 3)	1.83 ± 0.30 cm	0.965	0.927-0.983	<0.001	0.06 cm	0.11 cm	16.16 %
Inter-rater (EV 2 *VS* EV 3)	1.85 ± 0.35 cm	0.926	0.845-0.965	<0.001	0.09 cm	0.19 cm	18.85 %
Inter-analyst inter-rater	1.83 ± 0.30 cm	0.986	0.976-0.933	<0.001	0.04 cm	0.07 cm	16.34 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **IC:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation**. EV**: evaluator; ***VS***: versus.

Table 2.7. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for gastrocnemius lateralis muscle thickness (GL_{MT}).

Gastrocnemius Lateralis Muscle Thickness							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	1.34 ± 0.20 cm	0.959	0.913-0.980	<0.001	0.04 cm	0.08 cm	15.09 %
Inter-analyst intra-rater	1.31 ± 0.23 cm	0.931	0877-0.963	<0.001	0.06 cm	0.12 cm	14.24 %
Inter-rater	1.49 ± 0.20 cm	0.960	0.926-0.980	<0.001	0.04 cm	0.08 cm	13.39 %
Inter-rater (EV 1 *VS* EV 2)	1.32 ± 0.20 cm	0.953	0.902-0.978	<0.001	0.04 cm	0.09 cm	15.13 %
Inter-rater (EV 1 *VS* EV 3)	1.35 ± 0.20 cm	0.945	0.885-0.974	<0.001	0.05 cm	0.09 cm	14.53 %
Inter-rater (EV 2 *VS* EV 3)	1.34 ± 0.25 cm	0.924	0.841-0.964	<0.001	0.06 cm	0.11 cm	15.19 %
Inter-analyst inter-rater	1.30 ± 0.23 cm	0.935	0.890-0.965	<0.001	0.06 cm	0.12 cm	17.93 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **IC:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. **EV**: evaluator; ***VS***: versus.

Table 2.8. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for soleus muscle thickness (SO_{MT}).

Soleus Muscle Thickness							
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	1.48 ± 0.23 cm	0.980	0.958-0.990	<0.001	0.03 cm	0.06 cm	15.71 %
Inter-analyst intra-rater	1.44 ± 0.24 cm	0.954	0.938-0.981	<0.001	0.05 cm	0.10 cm	17.02 %
Inter-rater	1.48 ± 0.25 cm	0.972	0.948-0.986	<0.001	0.04 cm	0.08 cm	16.74 %
Inter-rater (EV 1 *VS* EV 2)	1.49 ± 0.24 cm	0.960	0.915-0.981	<0.001	0.05 cm	0.10 cm	16.32 %
Inter-rater (EV 1 *VS* EV 3)	1.48 ± 0.25 cm	0.945	0.885-0.974	<0.001	0.06 cm	0.12 cm	16.93 %
Inter-rater (EV 2 *VS* EV 3)	1.48 ± 0.25 cm	0.968	0.933-0.985	<0.001	0.05 cm	0.09 cm	16.02 %
Inter-analyst inter-rater	1.44 ± 0.25 cm	0.968	0.946-0.983	<0.001	0.05 cm	0.09 cm	17.63 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **IC:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. **EV**: evaluator; ***VS****:* versus.

Our muscle architecture inter-analyst comparisons for the intra-rater and for the inter-rater comparisons showed a high ICC (GM_{FL}: 0.845, 0.897; GL_{FL}: 0.766, 0.845; GM_{PA}: 0.927, 0.842; GL_{PA}: 0.894, 0.856; GM_{MT}: 0.979, 0.986; GL_{MT}: 0.931, 0.935; SO_{MT}: 0.954, 0.968). SEM values (GM_{FL}: 0.20cm, 0.26cm; GL_{FL}: 0.59cm, 0.52cm; GM_{PA}: 0.76º, 0.84º; GL_{PA}: 0.83º, 1.04º; GM_{MT}: 0.04cm, 0.04cm; GL_{MT}: 0.06cm, 0.06cm; SO_{MT}: 0.05cm, 0.05cm) and MDC values (GM_{FL}: 0.57cm, 0.51cm; GL_{FL}: 1.17cm, 1.02cm; GM_{PA}: 1.49º, 1.24º; GL_{PA}: 1.63º, 2.05º; GM_{MT}: 0.09cm, 0.07cm; GL_{MT}: 0.12cm, 0.12cm; SO_{MT}: 0.10cm, 0.9cm) were considered low in relation to the obtained mean values.

2.5 DISCUSSION

Due to its cost, effectiveness and accessibility, US has been used in several studies and in clinical settings to evaluate muscle architecture parameters. However, US is a technique that has been considered rater dependent (HARRIS-LOVE et al*.*, 2014). Therefore, the main purpose of the present study was to evaluate the US technique reliability in intra-rater, inter-rater and inter-analyst comparisons. Our results showed that US images can be obtained with a very high reliability in different moments by the same rater, by different raters and can be analyzed by different analysts.

Between the aforementioned comparisons, intra-rater is the most commonly found, with US measurements taken from different populations and different muscles (NARICI et al., 1996; RAJ et al., 2012; BARONI et al., 2013). In the present study, all ICC values obtained in the intra-rater comparisons were considered high (ICC > 0.90), demonstrating that these measures are highly reproducible when performed by the same rater at different times. Aggeloussis et al. (2010) and Giannkou *et al*. (2011) assessed the intra-rater reliability of the gastrocnemius medialis fascicle length and pennation angle during human gait. The coefficient of multiple correlations (CMC) ranged between 0.892 and 0.902 for pennation angle and 0.920 and 0.957 for fascicle length, resembling the ICC values found in the present study (GM_{FL}: 0.845; GM_{PA}: 0.927).

However, these studies evaluated the muscular architecture parameters during walking. No studies were found that evaluated the intra-rater reliability with the musculature at rest. Narici et al. (1996) assessed gastrocnemius medialis repeatability for fascicle length, pennation angle (mean of the superior and inferior pennation angles) and muscle thickness in healthy subjects at rest. The values found in their study were 5.1 ± 0.4 cm, 17.3 ± 2.6º and 2.0 ± 0.2 cm, respectively. In the present study, the gastrocnemius medialis intra-rater mean values for fascicle length, pennation angle and muscle thickness were 5.34 ± 0.64 cm, 22.13 ± 2.41º and 1.83 ± 0.29 cm. The intra-rater coefficient of variation values in the present study (GM_{FL}: 11.91%, GM_{PA}: 10.87%, GM_{MT}: 15.97%) were slightly higher than those of Narici *et al*. (1996) (GM_{FL}: 5.9%, GM_{PA}: 9.8%, GM_{MT}: 4.8%). A possible explanation for the coefficient of variation in the present study was that the sample consisted of men and women, whereas Narici et al., (1996) evaluated only men. Nevertheless, the high reliability found shows that these measures can be evaluated in different days by the same rater.

The GM_{MT} intra-rater ICC was 0.967. However, no studies were found that evaluated this specific muscle and presented intra-rater ICC calculations. Thus, it is not possible to compare this parameter with the previously reviewed literature. Nevertheless, the high reliability found here shows that GM_{MT} can be evaluated in different days by the same rater.

In the gastrocnemius medialis inter-rater comparisons, ICC values for fascicle length, pennation angle and muscle thickness measurements were also high (GM_{FL}: 0.920; GM_{PA}: 0.896; GM_{MT}: 0.970), with small SEM values (0.05 cm, 0.84º and 0.20 cm, respectively). These values were also similar to the study by KONIG *et al*. (2014). In this study, the observed ICC values were 0.77, 0.90 and 0.82 for GM_{FL}, GM_{PA}, and GM_{MT}, respectively. Additionally, the SEM values were 0.4 cm, 1.1º and 0.10 cm for the same variables. The relevance of a high inter-rater reliability can be exemplified by a scenario where a subject can be evaluated by different raters or clinicians, not depending on the times when the rater is available, and allowing a greater ease in conducting this evaluation in clinical settings (KONIG *et al*., 2014). However, one of the limitations in Konig *et al*. (2014) study was that inter-rater reliability assessment was determined for all participants by 2 raters independently. Kottner *et al*. (2011) presented the guidelines for reporting reliability and agreement studies, and they showed that reliability studies should be done with the maximum possible number of raters (minimum three raters).

The gastrocnemius medialis reliability results from some studies could be generalized for the gastrocnemius lateralis and soleus muscles, because they belong to the same muscle-tendon unit (AGGELOUSSIS *et al*., 2010; GIANNKOU *et al*., 2011). However, no study evaluated the intra and inter-rater reliability of the GL_{FL}, GL_{PA}, GL_{MT} and SO_{MT} measures, making comparisons impossible. Nevertheless, compared to the literature, the presented values seem to be valid. Fouré et al. (2013) evaluated the GL_{FL} and GL_{PA} measurements in healthy individuals, before and after a period of intervention. The mean values before training were 9.1 ± 3.1 cm, and 12.3 ± 2.4º, respectively. Stephensen et al. (2013) compared the GL_{FL}, GL_{PA}, and GL_{MT} measurements in healthy individuals with men with haemophilia. The healthy subjects mean values were 6.30 ± 0.20 cm, 15.83 ± 4.21º and 1.70 ± 1.50 cm, respectively. The mean intra-rater reliability values for GL_{FL}, GL_{PA} and GL_{MT} in the present study (4.41 ± 0.86 cm; 16.19 ± 2.36° and 1.34 ± 0.20 cm) were also similar to those by Fouré et al. (2013) and by Stephensen et al. (2013).

Reliability of the majority of the muscle architecture parameters, expressed by the ICC, was high. Only the GM_{PA} and GL_{PA} presented smaller values that were considered moderately high. Pennation angle is a very sensitive parameter that might be affected by the probe rotation and inclination during evaluation and is dependent on the parallel alignment of the deep aponeurosis with respect to superficial aponeurosis. In addition, especially for untrained raters, it is challenging to clearly visualize the muscle fibers, making their angulation calculation even more difficult. Therefore, the acquisition of the GM and GL muscles ultrasound images, from which the GM_{PA} and GL_{PA} parameters are obtained, must be performed with caution.

Another important factor for measuring muscle architecture parameters is data analysis, here performed in *Image-J* (National Institute of Health, USA) software by two different analysts. Inter-analysts comparisons of GL_{FL} and GL_{PA} presented lower ICC values compared to the other muscles. These low inter-analysts ICC values for GL_{FL} and GL_{PA} suggests that this parameter is directly influenced by whoever analyzes the images. This interpretation is reinforced by the low SEM values for GL_{FL} and GL_{PA} found in the present study (0.59cm, 0.52cm; 0.83º, 1.04º).

These results indicate that there is great US image reliability when analyzed by different analysts, suggesting that analysis can be safely done by different analyzers. No studies were found that evaluated inter-analyst reliability for plantar flexor muscles in healthy subjects. However, Sarwal et al. (2015) found similar values for quadriceps and abdominal muscles, with ICC values ranging from 0.84 to 0.99 while evaluating a critically ill population.

In this study, we followed the protocol from the studies previously found in the literature (KONIG et al., 2014, BRAFOD et al., 2018). To perform the inter-raters evaluations, raters were not present during the evaluations performed by the other evaluators, and all pen-markings on the skin were erased to not influence the next rater assessments. In addition, for the 30 evaluated participants, the evaluators' order was intentionally randomized, so that the evaluation order did not influence the results.

The identification of the probe position at the subjects' thigh was made through the distance measures between anatomical points, localized by palpation by each rater. Therefore, the raters' ability to identify the anatomical points also influenced the measurements reproducibility. This procedure was different from previous studies (e.g. BLAZEVICH et al., 2006) that used a map made from a transparent acetate sheet to position the probe in exactly the same way as in the first evaluation. This map is produced using anatomical points such as skin signs, scars and bony protrusions that are recorded as a reference. Although this map could have improved the reliability results by the usage of the same map by all raters, we preferred that reliability was based on the ability of the rater to perform the whole procedure by the positioning the US probe by each rater.

The raters' experience also plays a very important role in the reliability measurements (WEEKS et al., 2012), since experienced raters easily identify these anatomical points and the structures that need to be obtained during the US measurements evaluation. In the present study, the three raters had different levels of experience. While rater 1 had two years of experience with the technique assessment, rater 2 had one year of experience and rater 3 worked with the technique for only six months.

Despite this raters' experience difference, apparently the experience did not play a significant role in the US reliability results, as SO_{MT} measurements showed high reliability when we compared evaluators 2 and 3 (ICC: 0.968), 1 and 3 (ICC: 0.945), and 1 and 2 (ICC: 0.960). Similarly, for GM_{FL}, GL_{FL}, GM_{PA}, GL_{PA}, GM_{MT}, GL_{MT}, reliability was high when we compared evaluators 1 and 2 (ICCs: 0.948, 0.953, 0.865, 0.817, 0.974, 0.953, respectively), 1 and 3 (ICC: 0.854, 0.923, 0.847, 0.769, 0.965, 0.945), and 2 and 3 (ICC: 0.897, 0.902, 0.842, 0.783, 0.926, 0.924). These results suggest that six months of training seem to be sufficient to produce reliable results between different raters.

Similar results were found for the intra-rater reliability. ICC results were high when comparing evaluator 1 with himself for GM_{FL}, GL_{FL}, GM_{PA}, GL_{PA}, GM_{MT}, GL_{MT} and SO_{MT}, as the ICC values were 0.921, 0.978, 0.916, 0.970, 0.967, 0.959 and 0.980, respectively. These results further support that US measurements are reliable when performed by the same rater at different days. Nevertheless, it is fundamental that the raters have a proper training with the US technique before carrying out the evaluations, and also in order to know the assessment intrinsic variability. In addition, knowledge of the raters variability will assist researchers in indicating the true alteration of these US parameters and will allow for more accurate conclusions about these alterations causes (GIANNAKOU *et al*., 2011). For example, researchers will be able to know if the differences of muscle architecture parameters before and after training or other treatment were due to intervention or to biological variability and/or measurements' errors.

Finally, our results showed that US is a promising clinical tool for the assessment of Achilles tendon and plantar flexor muscles injuries. Our data can also be used as reliable data of a healthy condition for both men and women. These data from a normal healthy condition will also serve as the goal to be achieved in triceps surae injuries rehabilitation programs, which may have an impact on physiotherapists' clinical decision making. This should benefit researchers and practitioners who wish to know when a patient's score actually changed as a result from a rehabilitation program.

2.6 CONCLUSION

The high reliability observed for all intra-rater, inter-rater and inter-analyst US parameters demonstrates that these measurements are reliable for muscle architecture evaluation when performed by the same rater at different moments, and when performed by different raters and analysts. The bias that can influence these measurements must be identified, so its reliability can be increased in order to reduce possible measurement errors. Fascicle length reliability can be improved by standardizing the probe position. Pennation angle is biased by relatively high systematic and random error that arises from image interpretation. Further investigations are required to determine whether muscle architecture US is sensitive enough to track individual muscles adaptations.

2.7 REFERENCES

AAGAARD, P., ANDERSEN, J.L., DYHRE-POULSEN, P., LEFFERS, A.M., WAGNER, A., PETER MAGNUSSON, S., HALKJAER-KRISTENSES, J., SIMONSEN, E.B. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. **Journal of Physiology** (London), v.534, p.613-23, 2001.

ABELLANEDA, S., GUISSARD, N., DUCHATEAU, J. The relative lengthening of the myotendinous structures in the medial gastrocnemius during passive stretching differs among individuals. **Journal of Applied Physiology**, v.106, n.12, p.169-177, 2009.

AGGELOUSSIS, N., GIANNAKOU, E., ALBRACHT, K., ARAMPATZIS, A. Reproducibility of fascicle length and pennation angle of gastrocnemius medialis in human gait in vivo**. Gait & Posture**, v. 31, n.1, p. 73-7, 2010.

ARAMPATZIS, A., STAFIIDIS, S., DEMONTE, G., KARAMANIDIS, K., MOREYKLAPSING, G., BRUGEMANN, G.P. Strain and elongation of the human gastrocnemius tendon and aponeurosis during maximal plantarflexion effort. **Journal of Biomechanics**, v. 38, n.15, p.833-841, 2005.

BAPTISTA, R.R., VAZ, M.A. Muscle architecture and aging: functional adaptation and clinical aspects; a literature review. **Fisioterapia e Pesquisa**, v. 16, n.4, p.368-73, 2009.

BARFOD, K.W.; RIECKE, A.F.; BOESEN, A. et al. Validity and reliability of an ultrasound measurement of the free length of the Achilles tendon. **Danish Medical Journal,** v. 65, n. 3, p. 65-70, 2018.

BARONI, B.M., GEREMIA, J.M., RODRIGUES, R. et al. Muscle architecture adaptations to knee extensor eccentric training: rectus femoris vs. vastus lateralis. **Muscle and Nerve**, v. 48, n. 4, p. 498-506, 2013.

BARTLETT, J.W., FROST, C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. **Ultrasound Obstetrics Gynecology,** v. 31, n. 4, p. 466-75, 2008.

BIOLO, G., HEER, M., NARICI, M., STROLLO, F. Microgravity as a model of ageing. **Current Opinion in Clinical Nutrition & Metabolic Care**, v.6, n.1, p. 31-40, 2003.

BLAZEVICH, A.J., Effects of physical training and detraining, immobilisation, growth and aging on human fascicle geometry. **Sports Medicine**, v.36, n.12, p.1003-1017, 2006.

FINNI, T., HODGSON, J.A., LAI, A.M, EDGERTON, V.R, SINHA, S. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. **Journal of Applied Physiology**, v.95, n.16, p.829-837, 2003.

FOURÉ, A., NORDEZ, A., MCNAIE, P., CORNU, C. Effects of plyometric training on both active and passive parts of the plantarflexors series elastic component stiffness of muscle-tendon complex. **European Journal of Applied Physiology,** v.111, n. 15, p.539-548, 2011.

FRIEDERICH, J.A., BRAND, R.A. Muscle fiber architecture in the human lower limb. **Journal of Biomechanics**, v. 23, n.1, p.91-5, 1990.

GEREMIA, J.M.; BOBBERT, M.F.; CASA NOVA, M. et al. The structural and mechanical properties of the Achilles tendon 2 years after surgical repair. **Clinical Biomechanics**, v. 30, n. 5, p. 485-92, 2015.

GIANNAKOU, E., AGGELOUSSIS, N., ARAMPATZIS, A. Reproducibility of gastrocnemius medialis muscle architecture during treadmill running. **Journal of Electromyography and Kinesiology,** v.21, n.6, p.1081-6, 2011.

GILES, L.S., WEBSTER, K.E., MCCLELLAND, J.A., COOL, J. Does quadriceps atrophy exist in individuals with patellofemoral pain? A systematic literature review with meta-analysis. **Orthopaedics Sports and Physical Therapy,** v.43, n.11, p.766-776, 2013.

HARRIS-LOVE, M.O., MONAREDI, R., ISMAIIL, C., BLACKMAN, M.R, CLEARY, K. Quantitative Ultrasound: Measurement Considerations for the Assessment of Muscular Dystrophy and Sarcopenia. **Frontiers in Aging Neuroscience**, v.6, n.172, p. 168-172, 2014.

HOPKINS, W.G. Measures of reliability in sports medicine and science. **Sports Medicine**, v. 30, n.1, p.1-15, 2000.

KARAMANIDIS, K.; ARAMPATZIS, A. Mechanical and morphological properties of human quadriceps femoris and triceps surae muscle-tendon unit in relation to aging and running. **Journal of Biomechanics**, v. 39, n. 3, p. 406-417, 2006.

KAWAKAMI, Y., ABE, T., KUNO, S.Y., FUKUNAGA, T. Training induced changes in muscle architecture and specific tension. **European Journal of Applied Physiology and Occupational Physiology,** v.72, n.2, p. 37-43, 1995.

KAWAKAMI, Y., MURAOKA, Y., KUBO, K. SUZUKI, Y., FUKUNAGA, T. Changes in muscle size and architecture following 20 days of bed rest**. Journal of Gravitational Physiology,** v.7, n.3, p.53-59, 2000.

KIM, S.Y., BLEAKNEY, R.R., RINDLISBACHER, T., RAVICHANDIRAN, K., ROSSER, B.W., BOYNYON, E. Musculotendinous architecture of pathological supraspinatus: a pilot in vivo ultrasonography study. **Clinical Anatomy,** v.26, n. 2, p.228-235, 2012.

KOTTNER, J., AUDIGÉ, L., BROSON, S., DENNER, A., GAJEWSKI, B.J., HRÓBJARTSSON, A., ROBERTS, C., SHOUKRI, M., STREINEI, D.L. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. **Journal of Clinical Epidemiology,** v. 64, n.1, p.96-106, 2011.

KUBO, K., KANEHISA, H., AZUMA, K. et al. Muscle architectural characteristics in women aged 20-79 years. **Medicine and Science in Sports and Exercise**, v. 35, n. 1, p. 39-44, 2003a.

KONIG, N., CASSEL, M., INTZIEGIANNI, K., MAYER, F. Inter-rater reliability and measurement error of sonographic muscle architecture assessments. **Journal of Ultrasound in Medicine**, v. 33, n.5, p.769-777, 2014.

MEYER, D.C., GERBER, C., FARSHAD, M. Negative muscle pennation angle as a sign of massive musculotendinous retraction after tendon tear: paradoxical function of the vastus lateralis muscle. **Knee Surgery, Sports, Traumatology,** Arthroscopy, v. 19, n.9, p.1536-1539, 2011.

NARICI, M.V., BINZONI, T., HILTBRAND, E., FASEL, J., TERRIER, F., CERRETELLI, P. In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. **Journal of Physiology** (London), v. 496, n. 13, p.287-97, 1996.

NARICI, M. Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. **Journal of Electromyography and Kinesiology,** v. 9, n. 2, p. 97-103, 1999.

PORTNEY, L.E, WHATKINS, M.P. **Foundations of Clinical Research**: **Applications and Practice**, 3rd edition. Prentice Hall, New Jersey, 2009, p.595.

RAJ, I.S., BIRD, S.R., and SHIELD, A.J. Reliability of ultrasonographic measurement of the architecture of the vastus lateralis and gastrocnemius medialis muscles in older adults. **Clinical Physiology and Functional Imaging**, v. 32 n. 8, p. 65-70, 2012.

SARWAL, A., PARRT, S,M., BERRY, M.J., HSU, F.C., LEWIS, M.T., JUSTUS, N.W., MORRIS, P.E, DENEHY, L., BERNEY, S., DHAR, S., CARTWRIGHT, M.S. Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. **Journal of Ultrasound in Medicine,** v.34, n.7, p.1191-200, 2015.

SCHWENK, M., GOGULLA, S., ENGLERT, S., CZEMPIK, A., HAUER, K. Test-retest reliability and minimal detectable change of repeated sit-to-stand analysis using one body fixed sensor in geriatric patients. **Physiological Measurement**, v.33, n.11, p.1931-1946, 2012.

STEPHENSEN, D., SCOTT, O., DRECHSLER, W. Influence of ankle plantar flexor muscle architecture and strength on gait in boys with haemophilia in comparison to typically developing children. **Haemophilia**, v.12, n.1, p. 1-8, 2013.

VIM. **International Vocabulary of Metrology** – Basic and general concepts and associated terms (3rd ed.). Joint Committee for Guides in Metrology: 2012.

WARD, S.R., SARVER, J.J., ENG, C.M., KWAN, A., WURGLER-HAURI, C.C., PERRY, S.M., WILLIAMS, G.R., SOSLOWSKY, L.J., LIEBER, R.L. Plasticity of muscle architecture after supraspinatus tears. **The Journal of Orthopaedic and Sports Physical Therapy**, v. 40, n.11, p.729-735, 2010.

WALTER, S.D, ELIASZIW, M., DONNER, A. Sample size and optimal designs for reliability studies. **Estatistics in Medicine**, v.17, n.1, p.101-10, 1998.

WALKER, F.O, CARTWRIGHT, M.S, WIESLER, E.R, CARESS, J. Ultrasound of nerve and muscle. **Clinical Neurophysiology**, v. 115, n.3, p.495-507, 2004.

CHAPTER 3

INTRA-RATER, INTER-RATER AND INTER-ANALYST RELIABILITY OF ACHILLES TENDON MORPHOLOGICAL PROPERTIES IN HEALTHY INDIVIDUALS

Francesca Chaida Sonda, Emmanuel Souza da Rocha, Eliane Celina Guadagnin, Rodrigo Rabello da Silva, Jeam Marcel Geremia, Viviane Bortoluzzi Frasson, Marco Aurélio Vaz

3.1 INTRODUCTION

The assessment of Achilles tendon (AT) morphological properties has gained considerable attention due to its importance in human locomotion (KOMI et al., 1992) and high injury incidence (KUJALA et al., 2005). Previous in vivo techniques used to examine AT length and cross-sectional area (CSA) include magnetic resonance imaging (MRI), which is often considered the ‘‘imaging gold standard’’ (CHANG and MILER, 2009). However, MRI is often costly, time consuming and may not be readily accessible for many clinicians and researchers.

Ultrasonography (US) has gained popularity because of its ability to produce high-quality images of muscle and tendon morphology and is often considered to be safer (no ionizing radiation), relatively inexpensive and portable (CHANG and MILLER 2009; AHTIAINEN et al*;* 2010). However, it is an evaluator-dependent technique (MENDIS et al., 2010), which suggests that a large volume of practice to obtain the measures is fundamental and necessary before its use in clinical practice.

When considering a clinical application, it was shown that assessment of tendon morphology can identify the injury severity in tendon rupture patients (KONIG et al., 2014). Therefore, US examinations allow for a more comprehensive understanding of muscle-related disorders and injury mechanisms, and consequently might have an impact on clinical decision making (WARD et al., 2010; KIM et al., 2012).

To obtain meaningful information about patient-specific situations, the reliability of US evaluation has to be viewed in daily clinical routines. As the same patients are not always examined by the same clinician, different judgments of 2 or more evaluators have to be considered. Thus, the degree of reliability of its measurements depends on a strict protocol and knowledge of the analyzed structures by the examiner (KOTTNER et al., 2011).

Regardless of the device or protocol used in the functional assessment, it is essential for the methodology to be reliable so that the results are not influenced by the examiner or by the chronobiology (effect of the time). In other words, it is important that the variables measured are not affected by the number of intervening factors, since they would alter the precision of the measurements, what could lead to an excessive variability in the results (BARTLETT and FROST, 2008). This variability could hinder the determination of the measurements psychometric properties, as well as the between-studies results comparison (OLIVEIRA et al., 2012).

Reliability refers to the variation in measurements made on a subject under changing conditions. These conditions may be related to different assessment methods or instruments that are used (BARTLETT and FROST, 2008). Although the terminology is not constant between authors, and there are different kinds of reliability, three are commonly found in the literature. The intra-rater reliability compares the data collected by the same rater in different moments (WALTER et al., 1998, VIM, 2012). The inter-rater reliability compares the data collected by different raters in the same day (SEDREZ et al., 2018). The inter-analyst reliability uses images regardless of who collected them, comparing the analysis done by different analyzers (BARTLETT and FROST, 2008).

Previous studies evaluated the intra-rater reproducibility of these measures and presented, in general, good to excellent results for AT length (BARFOD et al., 2015; BROUWER et al., 2018), and CSA (BOHM et al., 2016) considering repeated measures with the individuals at rest and performing dynamic tasks. However, these studies were limited by lacking the inter-rater reliability evaluation and measures performed in different days. More recently, Barfod et al. (2018) evaluated AT free tendon length intra and inter-rater reliability in healthy individuals. They found a high intra and inter-rater ICC ($r>0.90$). Bohm et al. (2016) found good reliability for tendon CSA. However, this study evaluated only inter-rater reliability and the CSA was measured in the Achilles tendon most distal portion.

Thus, the researchers showed that the US method is not still sensitive enough to detect physiological changes or differences in AT CSA, as reported by longitudinal training intervention studies (ARAMPATZIS et al., 2007; BOHM et al., 2014). No studies were found that evaluated intra-rater, inter-rater and inter-analyst AT morphological properties reliability.

Morphological tendon properties are crucial pre requisite to investigate tendon plasticity in response to loading (FOURÉ et al., 2013), immobilization (KANNUS et al., 1997; GEREMIA et al., 2015) and pathologies (OLSSON et al., 2011). Considering the lack of evidence about the quantification of US assessment psychometric variables of the AT morphological properties, research concerning these aspects is still needed. Moreover, this information could serve as normative data for future comparison with other populations such as individuals with clinical, structural and/or functional alterations in the ankle joint. Furthermore, the use of a systematic and concise methodology for assessing the AT structure is necessary to improve the quality of the evaluations, allowing a better understanding of the structure, helping the rehabilitation process and permitting the development of injury prevention strategies.

3.2 PURPOSE

The main goal of the present study was to evaluate the intra-rater (measurements performed on different days by the same rater), inter-rater (measurements performed on the same day by three independent raters) and inter-analyst (images analyzed digitally by two different analysts) reliability of US Achilles tendon morphological properties. To accomplish this, we evaluated: (1) total tendon length, (2) free tendon length, (3) tendon cross-sectional area (CSA). The hypothesis raised in the present study was that all measures would have good reliability as an adequate training of the raters' team was carried out before the tests.

3.3 METHODS

The evaluations took place at the Neuromuscular Plasticity Department of the Exercise Research Laboratory (LAPEX, Porto Alegre, Brazil) of the Physical Education, Physiotherapy and Dance Faculty (ESEFID - UFRGS). This study used a methodology approved by the University's Research Ethics Committee (CAEE nº 79420817.6.0000.5347). Participants read and signed the informed consent form after they had all questions about the tests to be performed answered by the responsible researcher.

3.3.1 Sample

The sample size calculation was in accordance with the current literature that investigated the US reliability in the Achilles and patellar tendon (BARFOD et al., 2015, GELLHORN and CLARSON, 2013). The sample size was calculated with the G*Power software (Kiel European Journal of Applied Physiology 1 3 University, Germany). We assumed the null hypothesis value of ICC to be 0.40 (e.g. on the basis that any value lower than 0.40 might be considered clinically “unacceptable”); 80% of power; two replicated measurements (one for each evaluator or twice by the same evaluator); and a significance level of 95% to detect an ICC value of 0.70 based on previous literature (PORTNEY et al., 2009). A sample of 15 individuals was defined as the minimum number of subjects. However, an additional fifty percent of drop-out rate was included to consider those respondent(s) who would fail to attend the follow-up session (i.e. re-test). Furthermore, because we wanted to evaluate the measurements reliability in both sexes, 15 subjects from each sex were recruited totalizing 30 individuals.

3.3.2 Participants

Healthy and physically active university male (15) and female (15) subjects between 18-35 years old (25.6±4.2 years; height = 1.70±0.09 m; weight = 69.21±7.83 kg; all with right dominance) volunteered to participate in the study after disclosure of the project on the University's campus and by social networks. Participants were included if they: (1) had no lower or upper limb musculoskeletal injuries; (3) had no contraindication to perform maximal strength tests (e.g. cardiovascular, respiratory, neurological and musculoskeletal diseases); (4) had no difficulty to understand and/or execute the protocol tests; (5) did not miss any of the performed tests.

3.3.3 Experimental design

This study was characterized as a transversal correlational study, aimed at verifying intra, inter-rater, and inter-analyst reliability of Achilles tendon morphological properties.

After reading the consent form, participants were submitted to two assessment days separated by seven days. At the first session (1), the assessment was performed by evaluator 1, while at the second session (2) the same assessment was performed by evaluators 1, 2 and 3, successively (Figure 1).

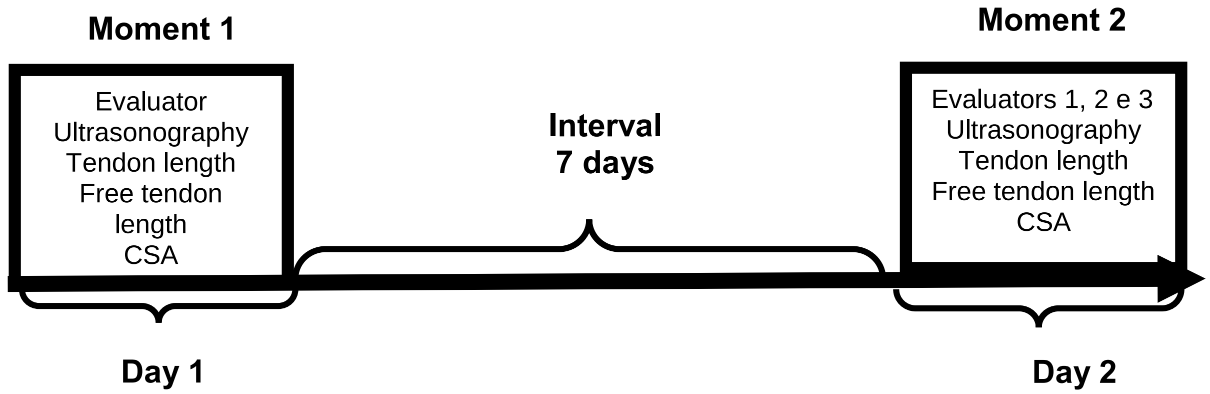


Figure 3.1. Experimental Design including intra and inter-rater reliability.

3.3.4 Procedures

Three evaluators with six months to two years of musculoskeletal US experience (evaluator 1 = two years; evaluator 2 = one year; evaluator 3 = six months) conducted all tests. They used the same methodology for all evaluations, and the evaluators order was randomly determined through the website www.random.org.

An additional researcher assisted the evaluators with the lab organization and the participant's positioning in each test. The three evaluators did not have contact during the procedures and were not in the lab during the data collection of the other two evaluators. The AT morphological properties of each participant was assessed through US, and each session lasted approximately 15 minutes.

3.3.5 Evaluation protocol

At the first evaluation, height and body mass were measured according to the International Society for Advancement of Kineanthropometry (MARFELL-JONES et al. 2006). Participants rested for 10 minutes before the US tests and were instructed not to engage in any vigorous physical activity for the previous 48 hours for each evaluation (BARONI et al., 2013). The Achilles tendon morphological properties were evaluated with a B-mode US system (LOGIQ P6, GE Healthcare, Waukesha, Washington, United States of America) and a linear matrix array transducer (50mm, 15 MHz - GE Healthcare, Waukesha, Washington, United States of America). The frequency was set to 11 MHz, a 3.5 cm depth and the focus was dynamically adjusted by the US operator. Great care was taken to determine the specific sites where the images were collected. Anatomical and reference points were marked on the skin. The reference-points were marked at the midpoint popliteal fold and at the lateral malleolus center. After each evaluation, the marks on the skin were removed in order to secure blinding between evaluators.

The images were obtained with the subjects in the ventral decubitus, with the knees fully extended (0°) and the ankle in neutral position (heel line at a 90° angle with respect to the longitudinal axis of the leg, 0° of plantarflexion). A custom system was used to secure the ankle in the neutral position (Figure 3.1). The ankle joint position was controlled using a digital goniometer (DIGIMESS, United States of America) (Figure 3.2).

Figure 3.2 Ankle in neutral position for morphological properties measurements.

Figure 3.3 Digital goniometer used to control the position of the ankle joint

The US probe was used in two distinct tendon orientations for the evaluation of tendon morphology: (1) in the sagittal plane, for evaluation of tendon length, and free tendon length and (2) in the transverse plane for obtaining the tendon cross-sectional area.

To obtain tendon length, the US probe was placed longitudinally to the tendon. The most distal portion of the Achilles tendon inserted into the calcaneus bone was determined by US and the respective point was marked on the skin (Figures 3.4 A and B). After this, the probe was moved to a proximal position until the visualization of the medial gastrocnemius myotendinous junction (MTJ), which was also marked on the skin (Figures 3.4 A and B). The distance between the two marked points on the skin was measured with a measuring tape (Figure 3.4 E), and this distance was considered representative of the tendon length (ARYA and KULIG, 2010; BARFOD et al., 2015; GEREMIA et al., 2018).

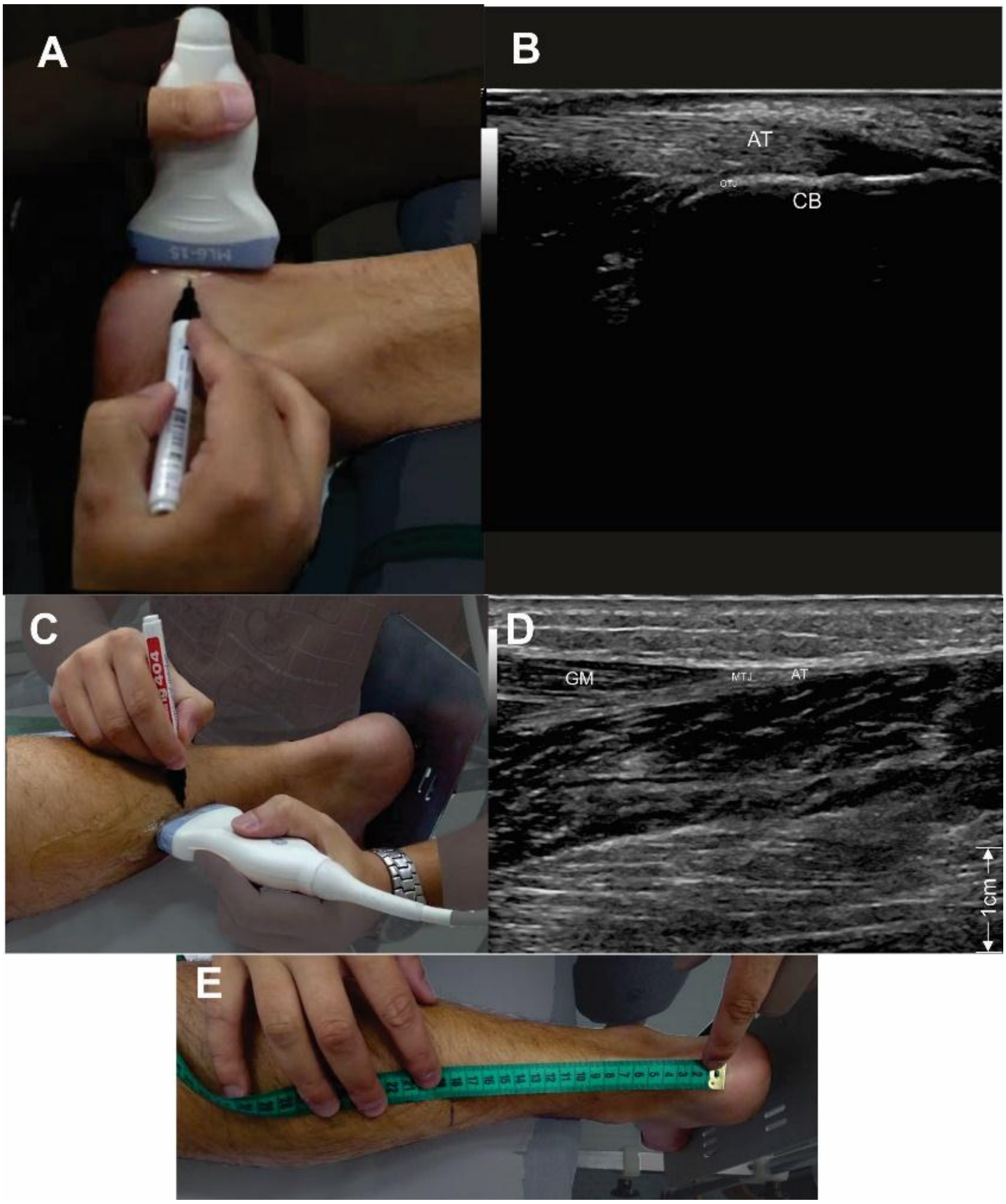


Figure 3.4 Tendon length. (A) The most distal portion of the Achilles tendon (AT) inserted into the calcaneus bone (CB) was marked on the skin; (B) Ultrasound image; (C) Myotendinous junction (MTJ) of the medial gastrocnemius muscle (GM) was marked on the skin; (D) Ultrasound image was used to locate the MTJ; (E) The distance between the two marked spots on the skin was measured with a measuring tape.

To obtain free tendon length, the US probe was placed longitudinally to the tendon. The most distal portion of the Achilles tendon inserted into the calcaneus bone was determined by US and the respective point was marked on the skin (Figures 3.5 A and B). After this, the probe was moved to a proximal position until the visualization of the soleus myotendinous junction (MTJ), which was also marked on the skin

(Figures 3.5 C and D). The distance between the two marked points on the skin was measured with a measuring tape (Figure 3.5 C), with this distance being considered representative of the free tendon length (BARFOD et al., 2018).

Figure 3.5 Tendon length. (A and B) Myotendinous junction (MTJ) of the soleus muscle was marked on the skin; (C) The distance between the two marked spots on the skin was measured with a measuring tape; (D) Ultrasound image was used to locate the MTJ.

To obtain the Achilles tendon cross-sectional area (CSA), the probe was placed perpendicular to the tendon (Figure 3.6 A and B), 3 images was obtained with reference to the distances of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 cm from the muscle insertion in the calcaneus bone (ARYA and KULIG, 2010). Area values were obtained for each image, and the final value of the area was calculated by the average of these five values.

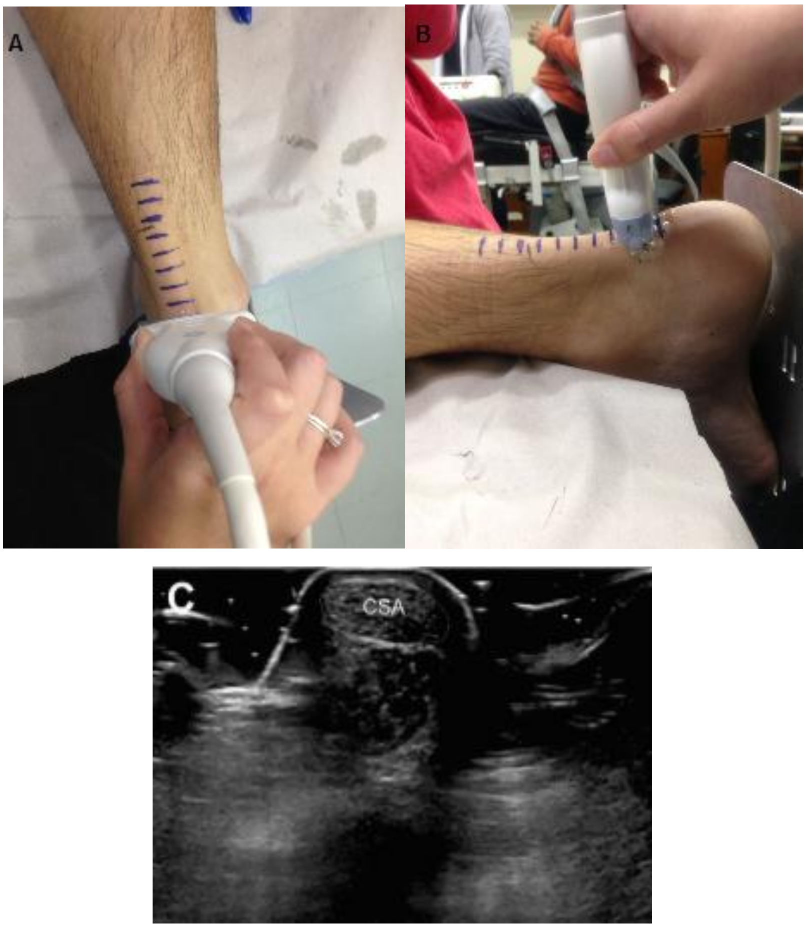


Figure 3.6 Measurement of the Achilles tendon cross-sectional area (CSA) using the ultrasound probe (A and B). Observe the skin marks every 1 cm (B) and an illustrative US image of how the CSA was obtained (C).

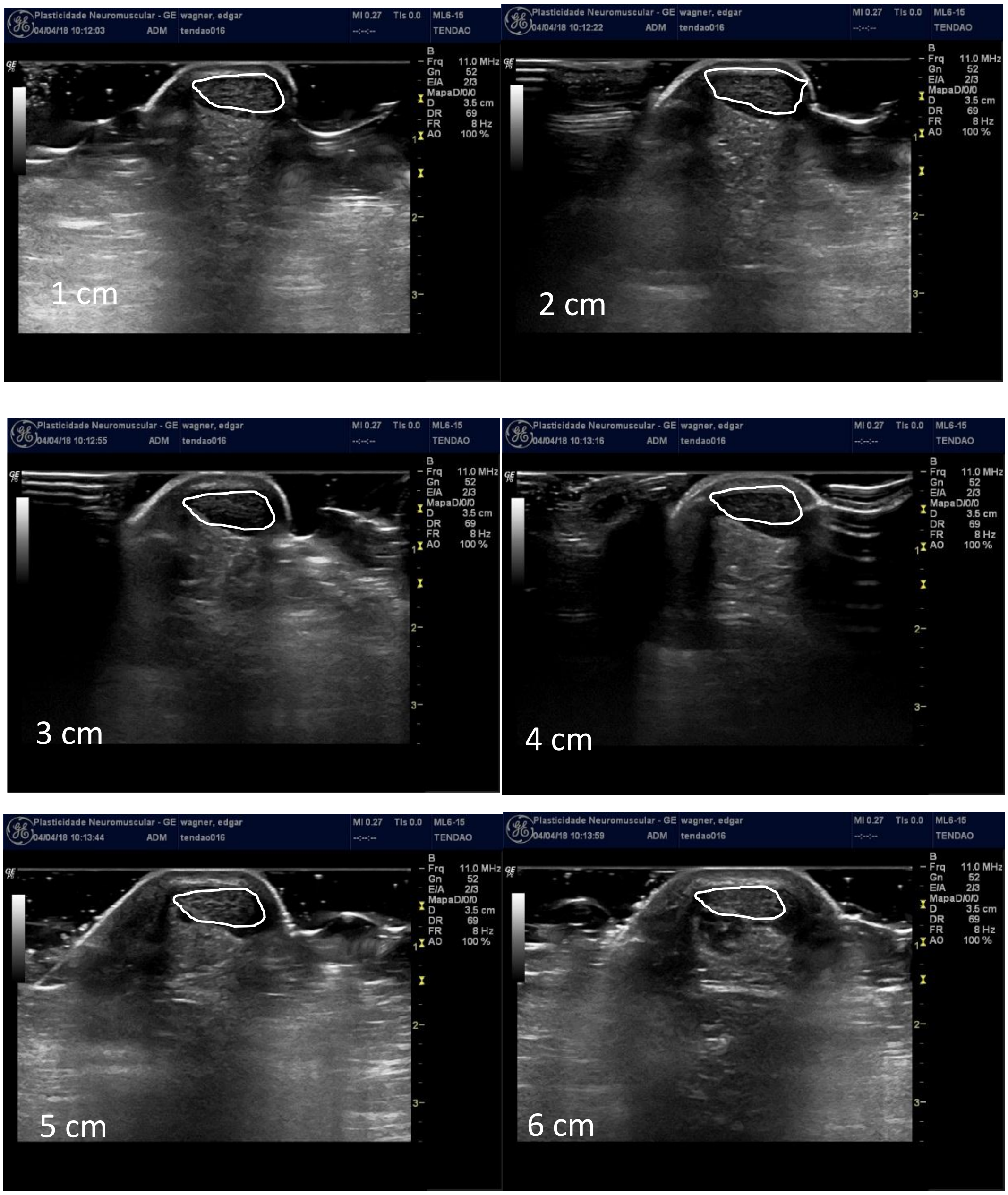
3.3.6 Data analysis

Achilles tendon CSA, was analyzed by two analysts with extensive experience. Blinding was achieved by a random-number code that was assigned to each image before testing and saved in a “master file”. This procedure was carried out by a research assistant.

During the tests, images were saved according to a random-number code to ensure that the image analysts were unaware of the identification of the images and which participants they represented. US images were digitized and analyzed with *ImageJ* version 1.46k23 software (Figure 3.7) (National Institutes of Health, Bethesda, Maryland).

For the measurement of Achilles tendon CSA, the image obtained with the US probe was analyzed by the image J software (National Institute of Health-NIH, USA). The CSA, in each of the 3 US images, was measured five times and the average in each of the positions was obtained. After this procedure, the Achilles tendon CSA was obtained using the following formula:

$$\mathbf{CSA} = \frac{\mathbf{CSA_{1cm} + CSA_{2cm} + CSA_{3cm} + CSA_{4cm} + CSA_{5cm} + CSA_{6cm} + CSA_{7cm} + CSA_{8cm} + CSA_{9cm} + CSA_{10cm}}}{\mathbf{10}}.$$



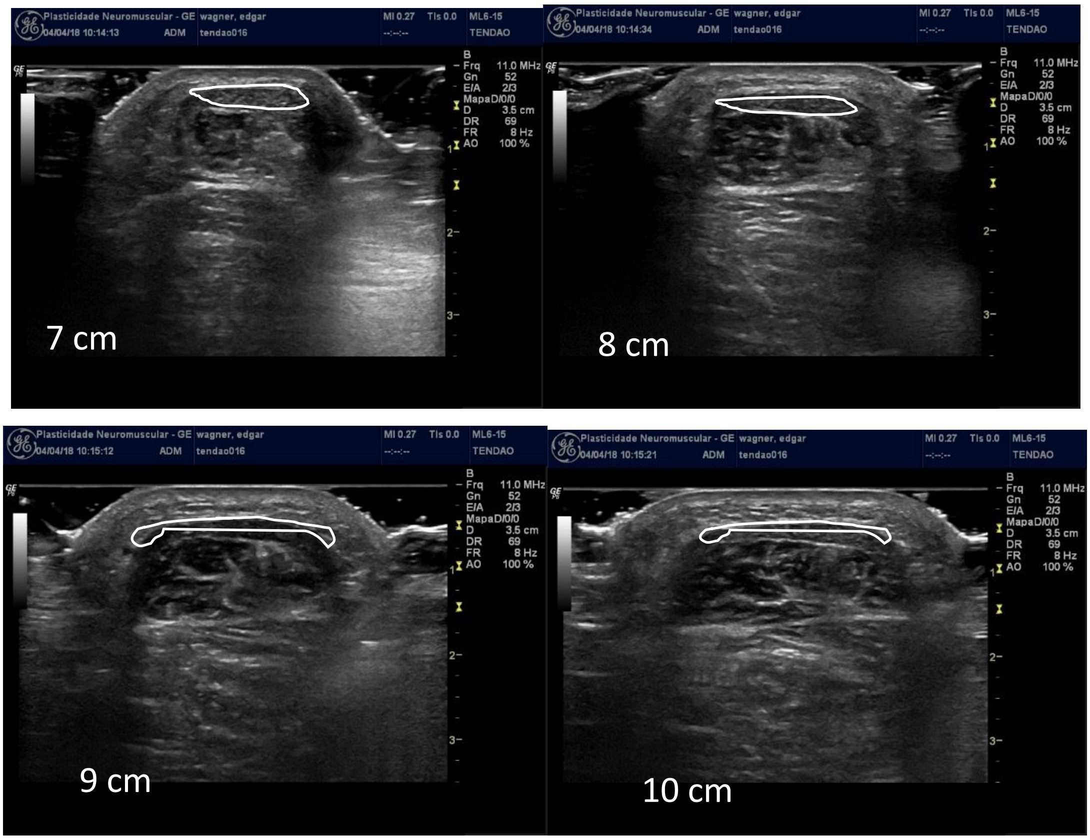


Figure 3.7 Analysis of the CSA data from 1 to 10 cm from the calcaneus.

3.3.7 Statistical Analysis

The statistical analysis was performed with the software SPSS 22.0. Initially, mean and standard deviation (SD) values were used in the descriptive analysis. The data normality was verified using the Shapiro-Wilk test. Values obtained by the same rater in different days were used to obtain the intra-rater reliability. Values obtained by different raters in the same day were used to obtain the inter-rater reliability. Values obtained by different analysts were used to obtain the inter-analyst reliability.

To verify the intra-, inter-rater and inter-analyst reliability, the ICC, the Standard Error of the Measurement (SEM), the Minimum Detectable Change (MDC), and the Coefficient of Variation (CV) were calculated. The ICC values were classified according to the literature (PORTNEY and WATKINS, 2009; SCHWENK et al., 2012) as weak (r<0.40), moderate (0.40 > r < 0.75), and excellent (r>0.75). The Standard Error of the Measurement (SEM) was estimated using the following equation: $SEM = SD * \sqrt{1 - ICC}$ where SD is the standard deviation of the measurements. The Minimum Detectable Change (MDC) was estimated based on a 95% confidence interval, where $MDC = 1.96 * SEM$. The coefficient of variation was calculated using the equation: CV=SD/X*100, where X is the mean value. A significance level of 5% was adopted for all analyzes.

The Bland and Altman (1986) analysis was used to illustrate the correlation between the measurements obtained by the different raters and was aimed at identifying possible bias and the limits of agreement (±1.96 standard deviations or the 95% confidence interval). The Student t test for independent samples was used to compare the difference between the different areas of interest. A horizontal line was drawn on the mean difference, and two additional lines at the limits of agreement (which are defined as the average difference of plus or minus 1.96 times the standard deviation of the differences, i.e., the confidence interval is 95%) with Office Excel (Microsoft, Redmond, WA, USA).

3.4 RESULTS

Mean ± SD values of ultrasound measures acquired in two different days by the same evaluator and in the same day by three different evaluators are presented in Table 3.1. The mean ± SD, ICC, confidence interval (CI), (SEM), (MDC), and (CV) for tendon length, free tendon length and CSA by the raters and analysts are presented in Tables 3.2, 3.3 and 3.4, respectively.

All parameters' intra-rater comparisons (tendon length, free tendon length and CSA) performed by the same evaluator on different days, showed a high ICC value (>0.75 – Table 3.2, 3.3 and 3.4). The SEM and MDC values for this comparison were low. Correlation values were significant for all comparisons ($p<0.001$).

In the inter-rater comparisons, the tendon length (Table 3) parameters showed high ICCs (0.901 to 0.957). The SEM and MDC values were low for measurement (0.40 cm to 1.69 cm). For the comparisons between raters, high ICC between rater 1 and 2 (0.957) was shown. The SEM and MDC values were low for tendon length between rater 2 and 3 (0.53 cm to 1.03 cm). Correlation values were significant for all comparisons ($p<0.001$).

In the inter-rater comparisons, the free tendon length (Table 3.4) parameters showed moderate ICCs (0.636). The SEM and MDC values were high (1.86 cm and 3.65 cm, respectively) in relation to the mean values. Comparisons between raters, showed low ICC between rater 1 versus rater 3 (0.372), and rater 2 versus rater 3 (0.293). Consecutively, SEM and MDC values were high (2.60 cm to 5.36 cm, respectively) in relation to the mean values.

In the inter-rater comparisons, the CSA (Table 3.4) parameters showed high ICCs (0.784 to 0.928). The SEM and MDC values were low for measurement (4.67 mm^2 to 17.17 mm^2). For the comparisons between raters, high ICC between rater 1 and 2 (0.928) was shown. The SEM and MDC values were low for tendon length between raters 2 and 3 (4.67 mm^2 to 9.16 mm^2). Correlation values were significant for all comparisons ($p<0.001$).

For inter-analyst comparisons of CSA measurements, ICC was high 0.911. Consecutively, SEM and MDC values were low (6.26 mm^2 and 12.28 mm^2, respectively) in relation to the mean values.

Bland-Altman plots revealed a relatively homogeneous dispersion of the tendon length, free tendon length and CSA values within the limits of agreement, indicating good agreement between the evaluators. (Figures 3.8, 3.9 and 3.10).

Table 3.1: Mean and standard deviation values of ultrasound measurements acquired in two different days by the same evaluator and in the same day by three different evaluators.

	Intra-Rater		Inter-Rater	
Ultrasound measurements	**1st Day** (Mean ± SD)	**2nd Day (Rater 1)** (Mean ± SD)	**Rater 2** (Mean ± SD)	**Rater 3** (Mean ± SD)
Tendon length (cm)	18.20 ± 2.03	18.27 ± 2.34	18.54 ± 2.75	20.35 ± 2.24
Free tendon length (cm)	5.31 ± 1.20	5.27 ± 1.31	5.38 ± 1.40	11.76 ± 1.76
CSA (mm^2)	60.39 ± 22.30	61.50 ± 16.25	62.60 ± 18.79	65.63 ± 19.10

Table 3.2. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for tendon length.

	Tendon length						
	Mean ± SD	**ICC**	**95% CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	18.24 ± 2.42 cm	0.973	0.944-0.987	<0.001	0.40 cm	0.78 cm	13.27 %
Inter-rater	19.05 ± 2.75 cm	0.952	0.911-0.975	<0.001	0.60 cm	1.18 cm	14.44 %
Inter-rater (EV 1 *VS* EV 2)	18.40 ± 2.54 cm	0.957	0.909-0.979	<0.001	0.53 cm	1.03 cm	13.79 %
Inter-rater (EV 1 *VS* EV 3)	19.31 ± 2.74 cm	0.901	0.792-0.953	<0.001	0.86 cm	1.69 cm	14.18 %
Inter-rater (EV 2 *VS* EV 3)	19.44 ± 2.87 cm	0.929	0.850-0.966	<0.001	0.77 cm	1.50 cm	14.77 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation; **EV**: evaluator; ***VS****:* versus.

Table 3.3 Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for free tendon length.

	Free tendon length						
	Mean ± SD	**ICC**	**95% CI**	**p-value**	**SEM (cm)**	**MDC (cm)**	**CV (%)**
Intra-rater	5.29 ± 1.25 cm	0.978	0.954-0.990	<0.001	0.18 cm	0.36 cm	23.55 %
Inter-rater	7.22 ± 3.08 cm	0.636	0.332-0.814	0.001	1.86 cm	3.65 cm	42.69 %
Inter-rater (EV 1 *VS* EV 2)	5.32 ± 1.34 cm	0.901	0.791-0.953	<0.001	0.42 cm	0.83 cm	25.24 %
Inter-rater (EV 1 *VS* EV 3)	8.15 ± 3.28 cm	0.372	-0.320-0.701	0.109	2.60 cm	5.10 cm	40.30 %
Inter-rater (EV 2 *VS* EV 3)	8.20 ± 3.25 cm	0.293	-0.485-0.664	0.178	2.74 cm	5.36 cm	39.68 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation; **EV**: evaluator; ***VS**:* versus.

Table 3.4. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation for CSA.

	CSA (mm²)						
	Mean ± SD	**ICC**	**95% CI**	**p-value**	**SEM (mm²)**	**MDC (mm²)**	**CV (%)**
Intra-rater	62.15 ± 17.71 mm²	0.986	0.843-0.964	<0.001	2.18 mm²	4.66 mm²	28.49 %
Inter-analyst intra-rater	57.17 ± 18.47 mm²	0.922	0.865-0.970	<0,001	5.16 mm²	10.11 mm²	32.31 %
Inter-rater	63.28 ± 17.98 mm²	0.893	0.803-0.945	<0.001	5.85 mm²	11.53 mm²	28.41 %
Inter-rater (EV 1 *VS* EV 2)	62.09 ± 17.41 mm²	0.928	0.849-0.966	<0.001	4.67 mm²	9.16 mm²	28.04 %
Inter-rater (EV 1 *VS* EV 3)	63.61 ± 17.72 mm²	0.829	0.641-0.919	<0.001	7.33 mm²	14.37 mm²	27.87 %
Inter-rater (EV 2 VS EV 3)	64.13 ± 18.85 mm²	0.784	0.546-0.897	<0.001	8.76 mm²	17.17 mm²	29.39%
Inter-analyst inter-rater	57.00 ± 21.00 mm²	0.911	0.850-0.952	<0.001	6.26 mm²	12.28 mm²	36.84%

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **CI:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation; EV: evaluator; ***VS****:* versus.

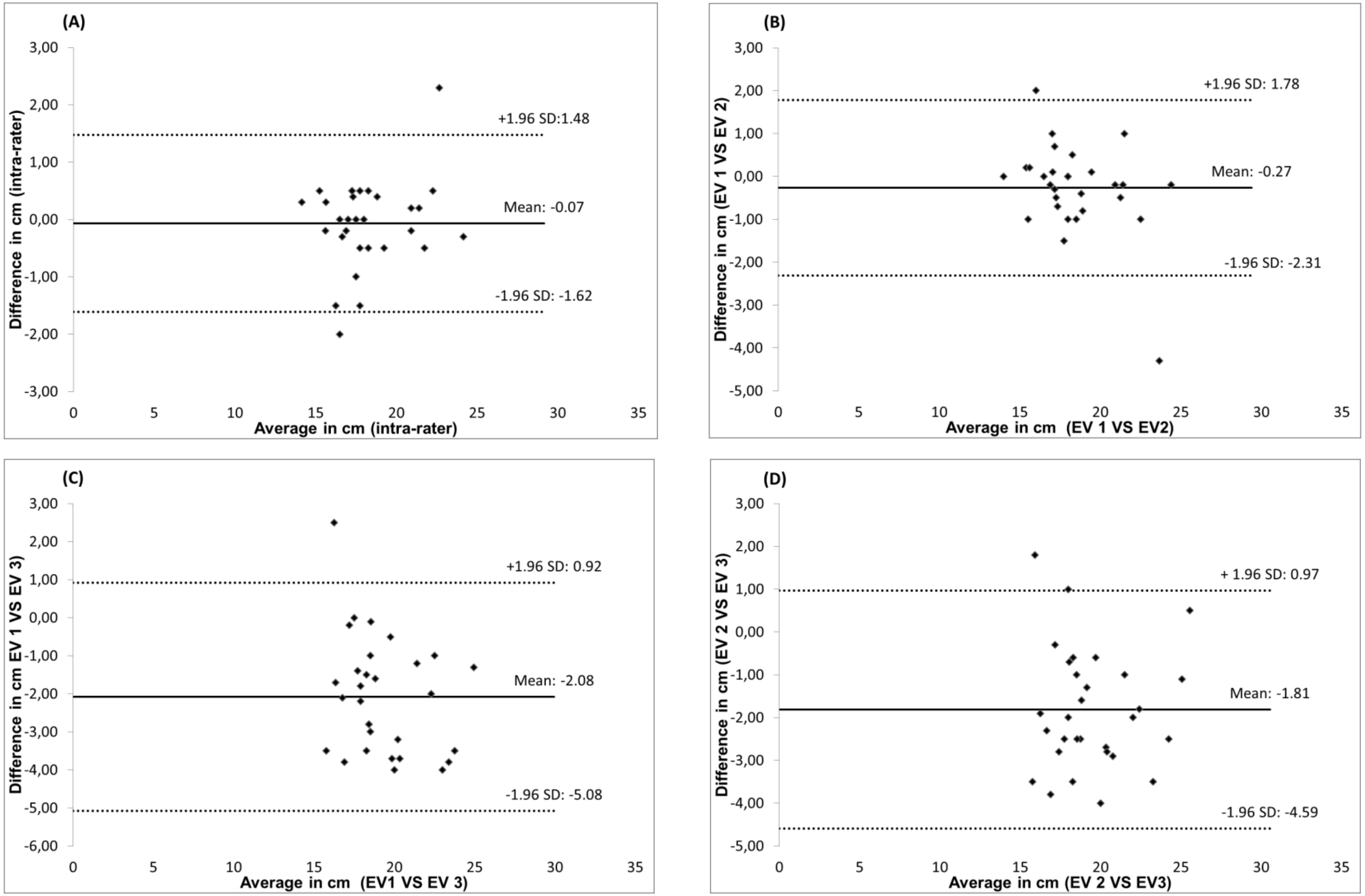


Figure 3.8: Bland-Altman method for tendon length between the evaluators: (a): intra-rater, (b) EV 1 VS EV 2, (c) EV 1 VS EV 3, (d) EV 2 VS EV3.

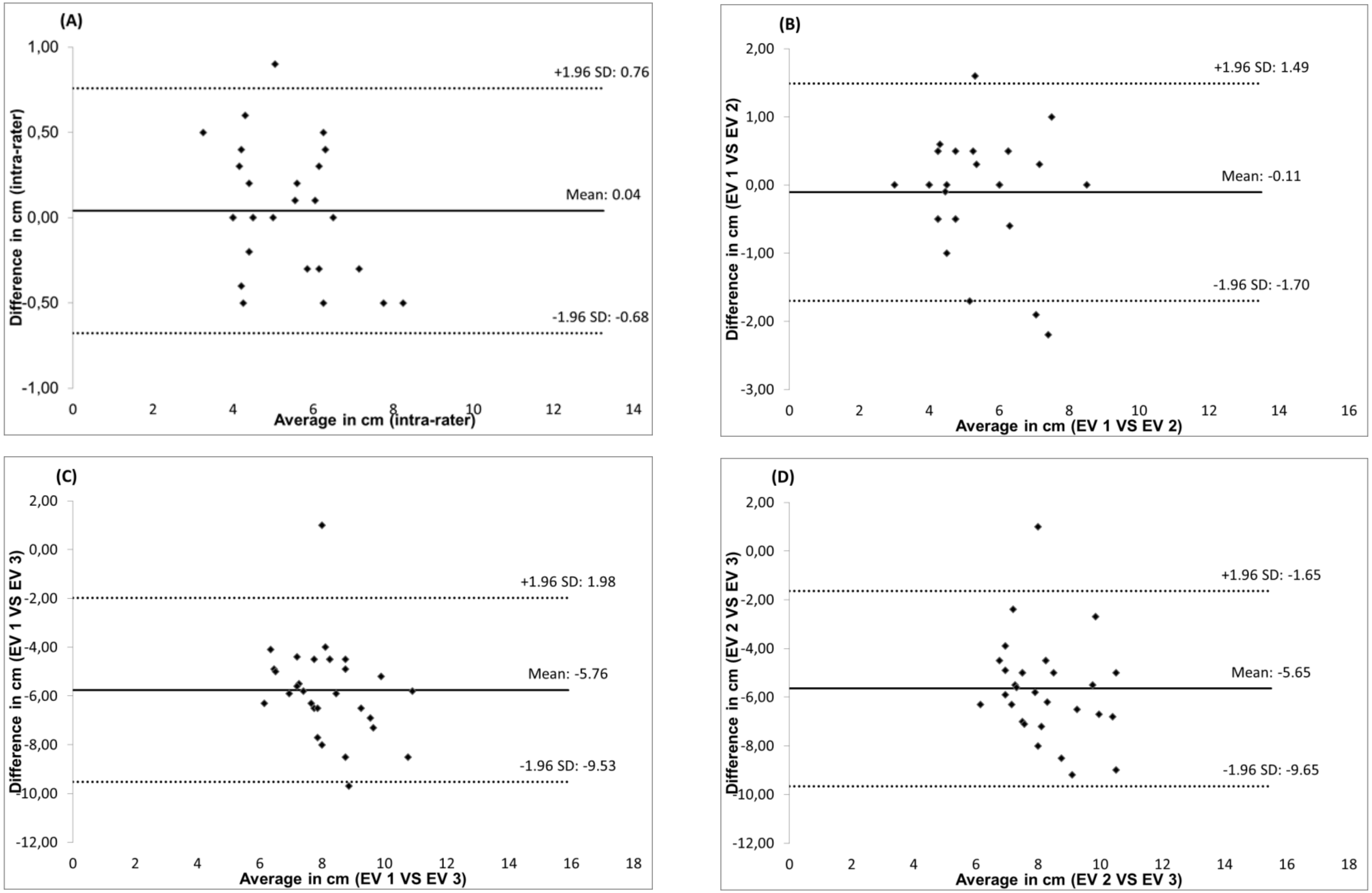


Figure 3.9: Bland-Altman method for free tendon length between the evaluators: (a): intra-rater, (b) EV 1 VS EV 2, (c) EV 1 VS EV 3, (d) EV 2 VS EV3.

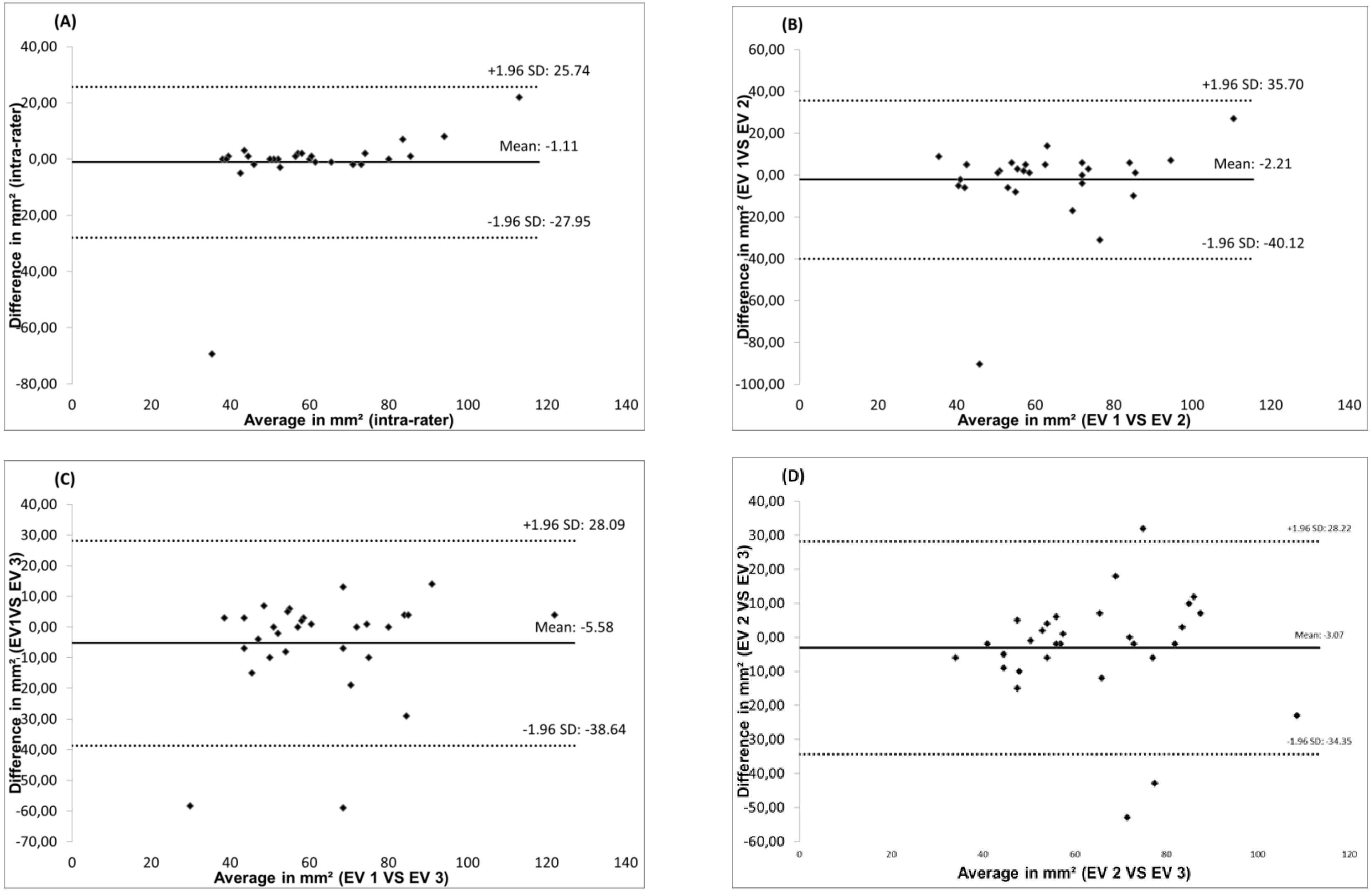


Figure 3.10: Bland-Altman method for CSA between the evaluators: (a): intra-rater, (b) EV 1 VS EV 2, (c) EV 1 VS EV 3, (d) EV 2 VS EV3.

3.5 DISCUSSION

The main purpose of the present study was to evaluate intra-rater, inter-rater and inter-analyst reliability of Achilles tendon morphology properties. All ICC values obtained in the intra-rater comparisons were considered high (ICC > 0.90), demonstrating that these measures are highly reliable when performed by the same rater at different times. In addition, the ICC values for tendon length, free tendon length and cross sectional area parameters in the intra-rater comparisons were almost identical to the values of previous studies (BARFOD et al., 2015; KRUSE et al., 2017; BARFOD et al., 2018).

While the present study found an ICC of 0.97, SEM of 0.40cm, MDC 0.78 cm for tendon length, previous studies evaluating the same parameter obtained ICC of 0.96 with SEM of 0.40, MDC of 1.0 cm (BARFOD et al., 2015) and ICC of 0.96 (BROUWER *et al*. 2018). For the free tendon length, the ICC score found was 0.97, SEM of 0.18cm, MDC of 0.36 cm. A value very similar to those found in the studies of Barfod et al. (2018), who found ICC of 0.94, SEM of 0.5 cm and MDC 1.3 cm for the same parameter, respectively. The ICC of the CSA was 0.98, similar to the other studies (BOHM et al., 2016; KRUSE et al., 2017). Therefore, the high reliability found shows that tendon length, free tendon length and CSA can be evaluated in different days by the same rater.

In the inter-rater comparisons, the tendon length ICC values (0.95), SEM (0.60cm) and MDC (1.18cm) were also similar to the studies previously found in the literature (BARFOD et al., 2015, BROUWER et al., 2018) with ICCs 0.97, 0.96, SEM of 0.3cm, 0.5cm and MDC of 0.9cm, 1.4cm, respectively. The most important finding of the present study was that AT length measurement showed good inter-rater reliability and in non-injured people. This is a measure that can be used to compare before and after treatment of Achilles tendon injuries and differences in tendon that suffered injury with healthy tendon. Additionally, tendon length can be used to document the magnitude and time course of Achilles tendon adaptations following a training program (GEREMIA et al., 2018). Therefore, this results showed that these measurement could to be assessed in the setting of patients with acute Achilles tendon rupture.

The inter-rater free tendon length measurements ICC was considered low (0.63) with an SEM of 1.86cm, MDC of 3.65cm. The only study evaluating this variable was the study of Barford et al. (2018), obtaining a high ICC of 0.96, SEM of 0.4cm, MDC of 1.1cm. Another explanation for the differences between raters could be transducer pressure applied during US measurements. Previous studies indicated that different degrees of transducer pressure could affect the AT morphological properties (DUDLEY-JAVOROSKI et al., 2010; MILGROM et al., 2014). In addition, these differences can be explained by the experience and level of training of the evaluators in this measure.

In the present study, the three raters had different levels of experience. While rater 1 had 2 years of experience with the assessment technique, rater 2 had one year of experience and rater 3 worked with the technique for only six months. When comparing the reliability obtained in comparisons between the most experienced rater and the other two raters individually, we observed that only the free tendon length was less reliable when we comparing the evaluator 1 with evaluator 3 (0.372) and when comparing evaluator 2 with evaluator 3 (0.293). However, when comparing evaluator 1 with evaluator 2, the ICC values were 0.901. Therefore, these differences may have been caused by the raters' different level of experience. Therefore, it is fundamental that the raters have a proper training with the technique before carrying out the evaluations. Moreover, it is advised that evaluators should be trained in the method and verify its reliability before starting the data collection, in order to know the variability intrinsic to its assessment (BARTLETT and FROST, 2008). Another factor that may have influenced this measure was that the measurement required visible marks to be made on the leg with a marker. The marks were removed between tests, but in some cases a vague mark persisted, which might have influenced the measurement of the next evaluators.

Caution should be exercised when applying and measuring free tendon measurement in patients who have ruptured the Achilles tendon. When interpreting the results, it is important to consider the morphology of the distal tip of the soleus, which might change after an Achilles tendon rupture (BARFOD et al., 2018).

Therefore, due to a low reliability found in our study, it is still not possible to apply this methodology in patients who suffered from tendinous rupture.

Particularly, care should be taken in the months following a rupture where the evaluators' experience is that the morphology of the distal tip of the soleus muscle is blurred and difficult to identify. Probably a measurement of the free part of the Achilles tendon, using the distal tip of the soleus muscle as a landmark, should not be performed until six months after injury in order for the landmark to become clear and easily identifiable (BARFOD et al., 2018).

For CSA measurements, ICC of 0.893, SEM of 5.85mm^2 and MDC 11.53 mm^2 was obtained in the inter-rater comparisons, a value considered high. Kruse et al. (2017) found high ICC values, but low values of SEM (0.22-1.47mm^2), MDC (0.94-4.07 mm^2) and CV (1.0-7.3%). Several other studies do not confirm our results and report moderate reliability values for an intra-rater assessment (i.e. ICC 0.79 (INTZIEGIANNI et al., 2015), ICC 0.76-0.92 (DUDLEY-JAVIROSKI et al., 2010), with limits of agreement of ±19% (BRUSHOJ et al., 2006)) as well as for a between-evaluator analysis (ICC 0.65 (DUDLEY-JAVOROSKI et al., 2010), with limits of agreement of ±19% (BRUSHOJ et al., 2006), demonstrating a common limitation of this method (BOHM et al., 2016). The limited consistency and the high variability in present study of the ultrasound-based Achilles tendon CSA values intra and inter-rater was caused by the unclear depiction of the tendinous tissue boundaries by the US imaging technique.

Furthermore, discrepancies in assessments were found for the proximal, medial and distal position, indicating a rather global deficit (i.e. independent of the tendon position) of the ultrasound-based method (BOHM et al., 2016). This study was the first to evaluate the AT CSA up to 10 cm from the tendon insertion, while other studies have evaluated up to 2 cm, 4 cm, 6 cm of insertion (ARYA and KULIG, 2010; GEREMIA et al., 2018). As a practical consequence, a comparison of CSAs provided by studies using different measurements is strongly affected by the differences inherent in the methodologies. Therefore, underestimated CSA strongly affect the calculation of Achilles tendon stress (i.e. tendon force divided by CSA) and the material properties (i.e. Young's modulus).

It is important to note that high measurement accuracy could only be achieved when the examination procedure is well standardized. In this context, a recent study (INTZIEGIANNI et al., 2015) agrees with our findings reporting good to excellent reliability for the AT CSA when the assessment was conducted at 4 and 6 cm proximal to the tendon insertion (ICC of 0.86 and 0.94; SEM of 4.4 and

2.9 mm2, respectively). The authors also provided the limits of agreement (15.5 and 11.9 mm², respectively). Although markers (metal wires) were used in that study, the joint angle was not controlled. Our study maintained control of the ankle joint in neutral position with a digital goniometer, and the markings were erased after the end of each evaluation.

Another important factor for measuring tendon morphology parameters is data analysis, here performed in *Image-J* (National Institute of Health, USA) software. Data analysis is challenging, especially when evaluating the AT CSA parameter, where the tendinous contour should be determined, excluding the aponeuroses. Our AT CSA inter-analyst comparisons showed a high ICC of 0.92, 0.91 followed by SEM (5.16mm², 6.26mm²) and MDC (10.11mm², 12.28mm²) values, which were considered low in relation to the obtained mean values. However, no studies were found that evaluated intra and inter-analyst reliability. Therefore, it is not possible to compare this parameter with the previously reviewed literature. These results indicate a great AT CSA images reliability when analyzed by different analysts, suggesting that analysis can be safely done by different analyzers. The only study that evaluated inter-analyzer reliability (KONIG et al., 2014) found values for gastrocnemius medialis, lateralis and soleus, finding ICC values ranging from 0.77 to 0.96 while evaluating healthy individuals.

Finally, the data produced in this study defined the MDC required for each of the dependent variables in order to consider whether changes on measurements are real. This should benefit researchers and practitioners who wish to know when a patient's score actually changed as a result from an intervention. Our data also can be used as normative reliable data of a healthy condition for both men and women when determining the goals to be achieved in rehabilitation programs of AT injuries.

3.6 CONCLUSION

The high reliability observed for all intra-rater parameters demonstrates that these measurements are accurate in the tendon morphology evaluation, when performed by the same rater at different moments. High reliability found for tendon length and CSA measures in inter-rater and inter-analyst comparisons demonstrates that these measures are also reliable in the evaluation, when performed by both different evaluators and analysts. The low reliability found for free tendon length in the inter-rater comparisons suggests that these parameters are evaluator-dependent, and that a longer period of training before data collection should be performed. Tendon length and CSA are a promising clinical tool to be further assessed in the setting of acute Achilles tendon rupture.

3.7 REFERENCES

AHTIANEN, J.P., HOFFREN, M., HULMI, J.J. et al. Panoramic ultrasonography is a valid method to measure changes in skeletal muscle cross-sectional area. European **Journal of Applied Physiology**, v.108, n.8, p. 273-279, 2010.

ARYA, S.; KULIG, K. Tendinopathy Alters Mechanical and Material Properties of the Achilles Tendon. **Journal of Applied Physiology,** v. 108, n. 3, p. 670-5, 2010.

ARAMPATZIS, A.; KARAMANIDIS, K.; ALBRACHT, K. Adaptational Responses of the Human Achilles Tendon by Modulation of the Applied Cyclic Strain Magnitude. **The Journal of Experimental Biology,** v. 210, n. 15, p. 2743-53, 2007.

BARFOD K.W., RIECKE, A.F, BOESEN, A. et al. Validation of a novel ultrasound measurement of achilles tendon length. **Knee Surgery, Sports Traumatology, Arthroscopy**, v.23, n. 11, p. 3398–3406, 2015.

BARFOD, K.W.; RIECKE, A.F.; BOESEN, A. et al. Validity and reliability of an ultrasound measurement of the free length of the Achilles tendon. **Danish Medical Journal,** v. 65, n. 3, p. 65-70, 2018.

BARONI, B.M., GEREMIA, J.M., RODRIGUES, R. et al. Muscle architecture adaptations to knee extensor eccentric training: rectus femoris vs. vastus lateralis. **Muscle and Nerve**, v. 48, n. 4, p. 498-506, 2013.

BARTLETT, J.W., FROST, C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. **Ultrasound Obstetrics Gynecology,** v. 31, n. 4, p. 466-75, 2008.

BLAND, J.M; ALTMAN D.G. Statistical methods for assessing agreement between two methods of clinical measurement. **Lancet**, v.9, n.1, p.307-10, 1986.

BROUWER, F.E., MYHRVOLD, S.B., BENTH, J.S et al. Ultrasound measurements of Achilles tendon length using skin markings are more reliable than extended-field-of-view imaging. **Knee Surgery, Sports Traumatology, Arthroscopy,** v. 26, n.7, p. 2088-2094, 2018.

BRUSHOJ, C., HENRIKSEN, B.M, ALBRECHT-BESTE, E. et al. Reproducibility of ultrasound and magnetic resonance imaging measurements of tendon size. **Acta Radiologica**, v. 47, n.9, p.954–959, 2006.

BOHM, S., MERSMANN, F., TETTKE, M. et al. Human Achilles tendon plasticity in response to cyclic strain: effect of rate and duration. **The Journal of Experimental Biology**, v. 217, n.22, p.4010-4017, 2014.

BOHM, S., MERSMANN, F., SCHROLL, A. et al. Insufficient accuracy of the ultrasound-based determination of Achilles tendon cross-sectional area. **Journal of Biomechanics,** v.49, n.13, p.2932-2937, 2016.

CHANG A., MILLER, T.T. Imaging of tendons. **Sports Health,** v.4, n.1, p.293–300.

DUDLEY-JAVOROSKI, S., MCMULLEN, T., BORGWARDT, M.R. et al. Reliability and responsiveness of musculoskeletal ultrasound in subjects with and without spinal cord injury. **Ultrasound in medicine & biology**, v. 36, n.10, 1595-1607, 2010.

FOURÉ, A., NORDEZ, A., MCNAIE, P., CORNU, C. Effects of plyometric training on both active and passive parts of the plantarflexors series elastic component stiffness of muscle-tendon complex. **European Journal of Applied Physiology,** v.111, n. 15, p.539-548, 2011.

GEREMIA, J.M., BOBBERT, M.F.; CASA NOVA, M. et al. The structural and mechanical properties of the Achilles tendon 2 years after surgical repair. **Clinical Biomechanics,** v. 30, n. 5, p. 485-92, 2015.

GEREMIA, J.M., BARONI, B.M., BOBBERT, M.F. et al. Effects of high loading by eccentric triceps surae training on Achilles tendon properties in humans. **European Journal of Applied Physiology**, v.118, n.8, p. 1725-1736, 2018.

GELHORN, A. C., CARLSON, M. J. Inter-Rater, Intra-Rater, and Inter-Machine Reliability of Quantitative Ultrasound Measurements of the Patellar Tendon. **Ultrasound and Medicine e Biology,** v.39, n.5, p. 791-796.

INTZIEGIANN K, CASSEL, M., KONIG, N., et al. Ultrasonography for the assessment of the structural properties of the Achilles tendon in asymptomatic individuals. An intra-rater reproducibility study. **Isokinetics and Exercise Science,** v.23, n.4, p.263-270, 2015.

HARRIS-LOVE, M.O., MONAREDI, R., ISMAIlL, C., BLACKMAN, M.R, CLEARY, K. Quantitative Ultrasound: Measurement Considerations for the Assessment of Muscular Dystrophy and Sarcopenia. **Frontiers in Aging Neuroscience**, v.6, n.172, p. 168-172, 2014.

HOPKINS, W.G. Measures of reliability in sports medicine and science. **Sports Medicine**, v. 30, n.1, p.1-15, 2000.

KANNUS, P., JOZSA, L., NATRI, et al. Effects of training, immobilization and remobilization on tendons. **Scandinavian Journal of Medicine & Science in Sports**, v. 7, n.2, p. 67–71.

KIM, S.Y., BLEAKNEY, R.R., RINDLISBACHER, T., RAVICHANDIRAN, K., ROSSER, B.W., BOYNYON, E. Musculotendinous architecture of pathological supraspinatus: a pilot in vivo ultrasonography study. **Clinical Anatomy,** v.26, n. 2, p.228-235, 2012.

KRUSE, A., STAFILIDIS, S., TILP, M. Ultrasound and magnetic resonance imaging are not interchangeable to assess the Achilles tendon cross‐sectional‐area. **European Journal of Applied Physiology**, v.117, n.1 p.73-82, 2017.

KOMI, P. **Strength and Power in Sport**. Vol 3. Encyclopaedia of Sports Medicine, 1992.

KONIG, N., CASSEL, M., INTZIEGIANNI, K., MAYER, F. Inter-rater reliability and measurement error of sonographic muscle architecture assessments. **Journal of Ultrasound in Medicine**, v. 33, n.5, p.769-777, 2014.

KOTTNER, J., AUDIGÉ, L., BROSON, S., DENNER, A., GAJEWSKI, B.J., HRÓBJARTSSON, A., ROBERTS, C., SHOUKRI, M., STREINEI, D.L. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. **Journal of Clinical Epidemiology,** v. 64, n.1, p.96-106, 2011.

KUJALA, U.M., SARNA, S., KAPRIO, J. Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. **Clinical Journal of Sport Medicine,** v.15, n.3, p.133-135, 2005.

MAFFULLI, N., ALMEKINDERS, LC. **The Achilles Tendon**. First Edition, *Springer*, 2007.

MARFELL-JONES, M., OLDS, T., STEWART, A., CARTER L. **International standards for anthropometric assessment***.* Potchefstroom, South Africa, ISAK 2006.

MENDIS, M. D.; WILSON, S. J.; STANTON, W. et al. Validity of Real-Time Ultrasound Imaging to Measure Anterior Hip Muscle Size: A Comparison with Magnetic Resonance Imaging. **The Journal of Orthopaedic and Sports Physical Therapy**, v. 40, n. 9, p. 577-81, 2010.

MILGROM, Y., MILGRON, C., ALTARAS, T. et al. Achilles tendons hypertrophy in response to high loading training. **Foot & Ankle International**, v.35, n.12, p.1303–1308, 2014.

PORTNEY, L.E, WHATKINS, M.P. **Foundations of Clinical Research**: Applications and Practice, 3rd edition. Prentice Hall, New Jersey, 2009, p.595.

OLIVEIRA, T. S., CANDOTTI, C. T., LA TORRE, M. et al. Validity and Reproducibility of the Measurements Obtained Using the Flexicurve Instrument to Evaluate the Angles of Thoracic and Lumbar Curvatures of the Spine in the Sagittal Plane. **Rehabilitation Research and Practice,** v.6, n.2, p.1-9, 2012.

OLSSON, N., NILSOON-HELANDER, K., KARLSSON, J. et al. Major functional deficits persist 2 years after acute Achilles tendon rupture. **Knee Surgery. Sports Traumatology, Arthroscopy**, v.19, n.8, p. 1385–1393.

SARWAL, A., PARRT, S,M., BERRY, M.J., HSU, F.C., LEWIS, M.T., JUSTUS, N.W., MORRIS, P.E, DENEHY, L., BERNEY, S., DHAR, S., CARTWRIGHT, M.S. Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. **Journal of Ultrasound in Medicine,** v.34, n.7, p.1191-200, 2015.

SCHWENK, M., GOGULLA, S., ENGLERT, S., CZEMPIK, A., HAUER, K. Test-retest reliability and minimal detectable change of repeated sit-to-stand analysis using one body fixed sensor in geriatric patients. **Physiological Measurement**, v.33, n.11, p.1931-1946, 2012.

VIM. **International Vocabulary of Metrology** – Basic and general concepts and associated terms (3rd ed.). Joint Committee for Guides in Metrology: 2012.

WARD, S.R., SARVER, J.J., ENG, C.M., KWAN, A., WURGLER-HAURI, C.C., PERRY, S.M., WILLIAMS, G.R., SOSLOWSKY, L.J., LIEBER, R.L. Plasticity of muscle architecture after supraspinatus tears. **The Journal of Orthopaedic and Sports Physical Therapy**, v. 40, n.11, p.729-735, 2010.

WALTER, S.D, ELIASZIW, M., DONNER, A. Sample size and optimal designs for reliability studies. **Statistics in Medicine**, v.17, n.1, p.101-10, 1998.

WALKER, F.O, CARTWRIGHT, M.S, WIESLER, E.R, CARESS, J. Ultrasound of nerve and muscle. **Clinical Neurophysiology**, v. 115, n.3, p.495-507, 2004.

CHAPTER 4

INTRA AND INTER-RATER RELIABILITY OF THE TRICEPS SURAE ISOMETRIC STRENGTH IN HEALTHY INDIVIDUALS

Francesca Chaida Sonda, Emmanuel Souza da Rocha, Eliane Celina Guadagnin, Rodrigo Rabello da Silva, Jeam Marcel Geremia, Viviane Bortoluzzi Frasson, Marco Aurélio Vaz

4.1 Introduction

Strength generated by muscles is responsible for joint torque production, which contributes to the development of human body movements and helps in joint stability and posture maintenance (HAMIL and KNUTZEN, 2009). Furthermore, strength evaluation enables the diagnosis of weakness as a consequence of muscular diseases and allows the quantitative assessment of functional recovery in training and rehabilitation programs (KANNUS et al., 1994; DYWER and DAVIS, 2008).

Currently, a widespread and commercially available method to measure muscle strength is the isokinetic dynamometer (KIM et al., 2014). Isometric tests using isokinetic dynamometers (BARONI et al., 2013) are commonly used in both clinical and academic activity. This equipment was introduced around the 1960s. One of the great advantages of isokinetic dynamometry is that it allows the assessment of the maximum (or submaximal) capacity of muscle strength throughout the articular range of motion at a constant velocity (IMPELLIZERI et al., 2008). In addition, it provides an accurate assessment of the torque, work and power of specific muscle groups (IMPELLIZERI et al., 2008). For this reason, it has been considered as the gold standard in the evaluation of musculoskeletal strength production capacity (LUND et al., 2005).

However, the usefulness of an isokinetic dynamometer depends upon the reproducibility, or reliability, of the equipment, the test protocol and the measurements obtained (ARNOLD et al., 1993). Reproducibility refers to the variation in measurements made on a subject under changing conditions. These conditions may be related to different assessment methods or instruments that are used (HOPKINS, 2000; BARTLETT and FROST, 2008). There is ample evidence that isokinetic tests of muscular strength are generally reliable (MORRIS-CHATA et al., 1994).

Many isokinetic reliability studies have focused on knee flexion and extension strength, and have shown the method to be highly reliable (ARNOLD et al., 1993; DROUIN et al., 2004; ALVARES et al., 2015). Moreover, a considerable number of studies have demonstrated high between-test reliability scores performed using the same isokinetic dynamometer (intra-machine reliability) (DIRNBERGER et al., 2012; ALVARES et al., 2015). These findings suggest, for example, that clinicians can have confidence in measurements to check patients' evolution throughout a rehabilitation program.

To the best of our knowledge, no previous study has assessed the intra and inter-rater reliability of isometric contractions in ankle plantarflexion strength. The strength of the triceps surae has an important role in locomotion, standing balance and many activities of daily living. For example, ankle plantar flexion power is a critical factor in modulating walking speed (LIU et al., 2008).

The assessment of ankle plantarflexion strength has received much attention due to the fact that Achilles tendon (AT) injuries have high incidence and are difficult to treat (COOK et al., 2002; LONGO et al., 2009). In addition, studies have shown, long time after surgery, that muscle strength is still reduced in patients who have ruptured their Achilles tendon (BRESSEL and MCNAIR, 2001; MAFFULLI et al., 2008).

Considering the lack of evidence about the quantification of psychometric variables of force production capacity of the plantar flexor muscles, research concerning these aspects is still needed. In addition, reliable measures of the maximum active plantar flexors torque are fundamental so that the mechanical properties and changes of these properties can be determined. Thus, the purpose of this study was to evaluate the triceps surae intra-rater (measurements performed on different days by the same rater), inter-rater (measurements performed on the same day by three independent raters) reliability of the isometric strength assessed on isokinetic dynamometer in healthy individuals.

4.2 METHODS

4.2.1 Participants

This study was characterized as a transversal correlational study and used a methodology approved by the University's Research Ethics Committee (CAEE nº 79420817.6.0000.5347), according to the declaration of Helsinki.

Healthy and physically active university male (n=15) and female (n=15) subjects between 18-35 years old (25.6±4.2 years; height=1.70±0.09 m; weight = 69.21±7.83 kg; all with right dominance) volunteered to participate in the study. Participants were included if they: (1) had no lower or upper limb musculoskeletal injuries; (3) had no contraindication to perform maximal strength tests (e.g. cardiovascular, respiratory, neurological and musculoskeletal diseases); (4) had no difficulty to understand and/or perform the protocol tests; (5) did not miss any of the performed tests.

Sample size calculation was in accordance with the current literature that investigated the plantar flexors' torque (GEREMIA *et al*., 2015). Sample size was calculated with the G*Power software (Version 3.1, Kiel University, Germany). We assumed the ICC null hypothesis value to be 0.40 (e.g. on the basis that any value lower than 0.40 might be considered clinically "unacceptable"); 80% of power; two replicated measurements and a significance level of 95% based on previous literature (PORTNEY *et al*., 2009). A sample of 16 individuals was defined as the minimum number of subjects. Furthermore, because we wanted to evaluate the measurements reliability in both sexes, were recruited a total of 30 individuals.

4.2.2 Procedures

Three evaluators with five years to two years by isokinetic dynamometry experience (evaluator 1 = five years; evaluator 2 = two years; evaluator 3 = two years) conducted all tests. They used the same methodology for all evaluations, and the evaluators order was randomly determined through the website www.random.org.

The evaluations took place in 2 different moments: day 1 and day 2 by evaluator 1 (7 days interval between data collections) and day 2 by evaluators 1, 2 and 3 in succession. On day 2, because each individual was submitted to three consecutive evaluations, a 30-min rest period was observed between each evaluator's tests in order to avoid fatigue.

The strength capacity of the triceps surae was obtained during maximal voluntary isometric ramping contractions (MVIRC). Participants were firmly strapped on the dynamometer, and were positioned with the subjects positioned with the hips in 90º of flexion, the evaluated limb with the knee fully extended and ankle in a neutral position, and the non-evaluated limb with knee and ankle relaxed. (Figure 4.1) and the ankle in the neutral position. The ramp protocol consisted of performing maximal torque production in a gradual manner, during 10 seconds (Figure 4.2). Subjects were instructed to do their maximal effort in a gradual manner.

Figure 4.1 Participant positioned on the isokinetic dynamometer for the isometric ramping contraction evaluations.

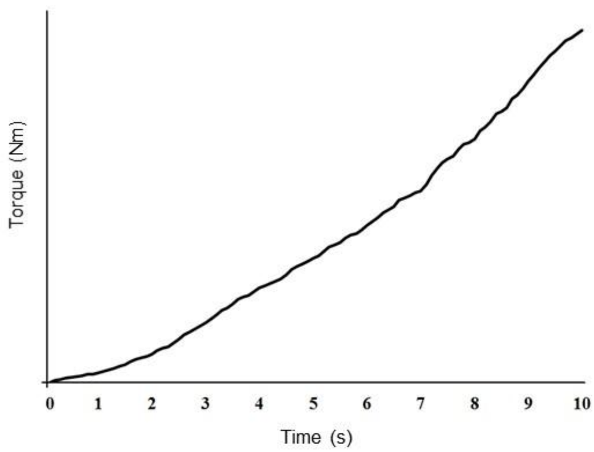


Figure 4.2 Ramp protocol. Individuals will have 10 seconds to achieve maximum torque gradually.

The warm-up protocol consisted of ten plantar flexion and dorsiflexion contractions in 120°•s^{-1}. Next, they performed the familiarization protocol, that consisted of three MVIRCs with 10 s of duration each, in a submaximal effort. After that, they performed the three MVIRCs in 10 s each, with maximal effort, considering a 2-min interval between consecutive contractions. The average of the three maximal contractions was used for the subsequent data analysis.

4.2.3 Statistical Analysis

The statistical analysis was performed with the software SPSS 22.0. Initially, mean and standard deviation (SD) values were used in the descriptive analysis. Data normality was verified using the Shapiro-Wilk test. Values obtained by the same rater in different days were used to obtain the intra-rater reliability. Values obtained by different raters in the same day were used to obtain the inter-rater reliability. To verify the intra- and the inter-rater reliability, the ICC, the Standard Error of the Measurement (SEM), the Minimum Detectable Change (MDC), and the Coefficient of Variation (CV) were calculated. The ICC values were classified according to the literature (PORTNEY and WATKINS, 2009; SCHWENK *et al*., 2012) as weak (r<0.40), moderate (0.40 > r < 0.75), and excellent (r>0.75). A significance level of 5% was adopted for all analyzes.

4.3 RESULTS

The mean ± SD values, ICC, confidence interval (CI), SEM, MDC, and CV for the plantar flexors torque for the raters are presented in Table 4.1.

All intra-rater comparisons showed a high ICC value (r > 0.80). The SEM and MDC values for this comparison were low. Correlation values were significant for all comparisons (p<0.001).

The inter-rater comparisons showed high ICCs (r > 0.80). The SEM and MDC values were low (11.75 N/m, 23.03 N/m). For the between-raters comparisons, showed high ICCs between raters 1 and 3 (r=0.927). The SEM and MDC values were low (9.64 N/m, 18.60 N/m). Correlation values were significant for all comparisons (p<0.001).

Table 4.1. Mean and standard deviation values, intraclass correlation coefficient, 95% confidence interval, standard error of measurement, minimum detectable change and coefficient of variation of plantar flexor torque.

	Plantar flexors torque						
	Mean ± SD	**ICC**	**95%CI**	**p-value**	**SEM (N/m)**	**MDC (N/m)**	**CV (%)**
Intra-rater	150.64 ± 36.49 N/m	0.873	0.734-0.940	<0.001	13.9 N/m	25.49 N/m	24.23 %
Inter-rater	150.33 ± 35.42 N/m	0.890	0.799-0.944	<0.001	11.75 N/m	23.03 N/m	23.56 %
Inter-rater (EV 1 *VS* EV 2)	150.00 ± 35.93 N/m	0.807	0.595-0.908	<0.001	15.79 N/m	30.94 N/m	23.96 %
Inter-rater (EV 1 *VS* EV 3)	150.58 ± 35.69 N/m	0.927	0.846-0.965	<0.001	9.64 N/m	18.90 N/m	23.70 %
Inter-rater (EV 2 *VS* EV 3)	150.41 ± 34.93 N/m	0.911	0.812-0.957	<0.001	10.42 N/m	20.42 N/m	23.22 %

SD: Standard deviation**; ICC:** intra-class correlation coefficient; **IC:** Confidence Interval; **SEM**: Standard error of measurement; **MDC**: minimum detectable change; **CV**: coefficient of variation. **EV**: evaluator; ***VS****:* versus.

4.4 DISCUSSION

The main purpose of the present study was to evaluate intra and inter-rater reliability of healthy individuals' triceps surae isometric strength in isokinetic dynamometer. Our results showed that the values found can be obtained with a very high reliability in different moments by the same rater and different raters.

In the present study, both intra- and inter-rater comparisons of force evaluations were considered to be highly reliable, with higher values observed for intra-rater comparisons. Kongsgaard et al. (2011) examined the Achilles tendon mechanical properties and assessed the between-day reproducibility of these measurements. The values found in their study were 192.4±22.9 N/m (day 1), and 201.1±66.7 N/m (day 2). In the present study, the mean values for plantar flexor torque were 154.43±36.82 N/m (day 1), and 146.84±36.38 N/m. However, Kongsgaard et al. (2011) did not calculate ICC, SEM and MDC values. Thus, it is not possible to compare this parameter with the previously reviewed literature. Nevertheless, the high reliability found shows that the triceps surae strength capacity can be evaluated in different days by the same rater.

No studies were found that evaluated the inter-rater reliability of the isometric strength of the triceps surae. In the present study, both intra- and inter-rater comparisons of force evaluations were considered to be highly reliable, with higher values observed for inter-rater comparisons. ICC values were 0.890 for inter-rater comparisons. This difference in values of intra and inter ICC values can be explained by the seven-day interval between evaluations. In addition, in the second evaluation, the participants were already familiar with the ramp protocol movement.

The raters' experience also plays a very important role in the reliability of the measurements. In the present study, the three raters had different levels of experience. While rater 1 had five years of experience with the isokinetic dynamometer, raters 2, and 3 had two years of experience. When comparing the reliability obtained in comparisons between the most experienced rater and the other two raters individually, we observed that they were high reliable when we compared the evaluator 1 with the evaluator 3 (0.927) and when comparing evaluator 2 with evaluator 3 (0.911). However, when comparing evaluator 1 with himself, the ICC values were 0.873. So, these differences may have been caused by the raters' different level of experience. Therefore, it is fundamental that the raters have a proper training with the technique before carrying out the evaluations. However, the high reliability between evaluators shows that this technique can be performed by evaluators who have less experience in the measurement.

When considering this type of evaluation, several potential sources of error in the test protocol have to be recognized and their effects reduced to optimize the reliability. Subject familiarization with the equipment is necessary and a pre-test should be done prior to the first test to facilitate the movements' coordination of the ankle joint (HOLMBACK et al., 1999). Additionally, the warm-up procedures before each test session in the present study followed a strictly standardized protocol with cycling and submaximal contractions at specific angular velocities.

Great care has to be taken to correctly position and stabilize the subject and the joint tested. Andersen et al. (1996) found that a 1.5 cm displacement of the ankle joint anatomic axis caused a 10% change in dorsiflexion and plantar flexion isokinetic peak torque and work. Oberg et al. (1987) showed that torques during isokinetic and isometric ankle strength testing were significantly higher without upper trunk fixation. In this study, positioning and fixation followed the standard procedures in the Biodex manual, and the position of the subject and the ankle joint were recorded during the first test and carefully reproduced with the other evaluators.

The reliability during the testing can be influenced by the interval between contractions, and between test sessions (STRATFORD et al., 1990) Andersen et al. (1996) found that a short rest between contractions resulted in higher isokinetic recordings than with no rest. No study has systematically addressed the effects of a rest period between trials, but it is likely that a rest will have a similar beneficial effect as a rest between contractions. In our study, the subjects rested 2 minutes between each maximal contraction and 30 minutes between evaluators. This was considered to be sufficient to reduce any effects of muscle fatigue on the measurements.

The time between test sessions varied considerably in previous studies of ankle dorsiflexion reliability. The shortest reported time has been 10 minutes in a study that showed low reliability (WENNERBERG et al., 1991). More commonly, 24 hours up to 7 days have elapsed between sessions (KIM et al., 2014), and these studies have generally found higher reliability. To ensure that the effects of both learning and fatigue were eliminated, the retest in this study was performed 7 days after the first test session.

It should be noted that any reliability study also has to take into account the characteristics of the subject being examined, such as their age and physical activity level, as well as their present and previous medical history (MORRIS-CHATA et al., 1994). The reliability results presented in this study are therefore only applicable to younger healthy men and women.

As in most clinical trials, the impossibility of guaranteeing that the subject performs the exact same amount of force in tests performed in both dynamometers is present in the present study. Despite our concern in trying to minimize this intervening variable through standardized instructions prior to testing and the continuous verbal encouragement during testing, there is no guarantee that volunteers have identical performance in the two evaluation sessions. The variation comes from several sources, and the main source is usually biological (ALVARES et al., 2015). An individual's maximum power output changes between trials because of changes in mental or physical state.

The measurement of muscle strength through isometric contractions proved to be an objective and useful tool for assessing ankle plantar flexor strength in a clinical environment. Therefore, evaluating the intra- and inter-rater reliability of dynamometry tests during a standardized performance was fundamental, as there is a need for precision in the measurements performed. Understanding reliability by means of voluntary muscle contractions may contribute to future studies in the selection of these variables. The identification of variables that show less reliability should lead to new procedures to improve their reliability or to their exclusion as an outcome variable if reliability cannot be improved.

Finally, our results showed that isometric ankle plantar flexion tests performed on the isokinetic dynamometer are a promising clinical tool for the assessment of Achilles tendon and plantar flexor muscles injuries related to strength output. Our data can also be used as normative reliable data of a healthy condition for both men and women. These data from a normal healthy condition will also serve as the goal to be achieved in triceps surae injuries rehabilitation programs, which may have an impact on clinicians decision making.

4.5 CONCLUSION

The high reliability observed for all intra-rater and inter-rater tests, indicates that these measurements are reliable to assess the triceps surae strength production capacity by the same rater at different moments, and when performed by different raters. These and other factors are present in the isokinetic assessment performed at research laboratories, sports clubs and rehabilitation clinics. Therefore, we believe that our findings depict the real situation that professionals involved in the human movement sciences experience in their daily practice.

4.6 REFERENCES

ALVARES, J.B.A.R, RODRIGUES, R., FRANKE, R.A. et al. Inter-machine Reliability of the Biodex and Cybex Isokinetic Dynamometers for Knee Flexor-Extensor Isometric, Concentric and Eccentric Tests. **Physical Therapy in Sport**, v. 16, n.1, p. 59-65, 2015.

ANDERSEN, H. Reliability of isokinetic measurements of ankle dorsal and plantar flexors in normal subjects and in patients with peripheral neuropathy. **Archives of Physical Medicine and Rehabilitation**, v 77 n.3, p. 265–268, 1996.

ARNOLD, B.L., PERRIN, D.H., HELLWING, E.V. The Reliability of Three Isokinetic Knee-extension Angle-specific Torques. **Journal of Athletic Training**, v.28, n.3, p.227-229, 1993.

BARTLETT, J.W., FROST, C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. **Ultrasound Obstetrics Gynecology,** v. 31, n. 4, p. 466-75, 2008.

BARONI, B. M.; RODRIGUES, R.; FRANKE, R. A. et al. Time Course of Neuromuscular Adaptations to Knee Extensor Eccentric Training. **International Journal of Sports Medicine,** v. 34, n. 10, p. 904-11, 2013b.

BRESSEL, E., MCNAIS, P. Biomechanical behavior of the plantar flexor muscle-tendon unit after an Achilles tendon rupture. **The American Journal of Sports Medicine**, v. 29 n.3, p. 321–326, 2001.

COOK, J.L., KHAN, M.N., PURDAM, C. Achilles tendinopathy. **Manual Therapy**, v. 7, n.3, p.121-130, 2002.

DIRNBERGER, J., KOSTERS, A., MULLER, E. Concentric and eccentric knee 5 extension: A reproducibility study using the IsoMed 2000-Dynamometer. **Isokinetics and Exercise Science**, v. 20, n. 8, p. 31-35, 2012.

DROUIN, J.M., VALOVICH-MCLEOD, T.C., SHULTZ, S.J. et al. Reliability and validity of the Biodex system 3 pro isokinetic dynamometer 9 velocity, torque and position measurement. **European Journal of Applied Physiology**, v. 91, n.13, p. 22-29, 2004.

DWYER, G.B., DAVIS, S.E. **American College of Sport Medicine**. ACSM's Health-Related Physical Fitness Assessment Manual. (2nd ed). Lippincott Willians & Wilkins, 2008.

GEREMIA, J.M.; BOBBERT, M.F.; CASA NOVA, M. et al. The structural and mechanical properties of the Achilles tendon 2 years after surgical repair. **Clinical Biomechanics,** v. 30, n. 5, p. 485-92, 2015.

HAMIL, J., KNUTZEN, K.M. **Biomechanical Basis of Human Movement**. 3rd Edition. Lippincott Williams & Wilkins, 2009.

HOLMBACK, A.M, POTER, M.M; DOWNHAM, D. et al. Reliability of isokinetic ankle dorsiflexor strength measurements in healthy young men and women. **Scandinavian Journal of Rehabilitation Medicine**, v.31, n.4, p. 229-239, 1999.

HOPKINS, W.G. Measures of reliability in sports medicine and science. **Sports Medicine**, v. 30, n.1, p.1-15, 2000.

IMPELLIZZEERI, F.M., BIZZINI, M., RAMPININI, E. et al. Reliability of isokinetic strength imbalance ratios measured using the Cybex NORM dynamometer. **Clinical Physiology and Functional Imaging**, v. 28, n.7, p.113-119, 2008.

KANNUS, P. Isokinetic evaluation of muscular performance: implications for muscle testing and rehabilitation. **International Journal of Sports Medicine**, v.15, n.1, p11-18, 1994.

KIM, S.Y., KO, J.B., FARTHING, J.P. et al. Investigation of supraspinatus muscle architecture following concentric and eccentric training. **Journal of Science and Medicine in Sport,** v.18, n.4, p.378-382, 2014.

KOONGSGAARD, M., NIELSEN, C.H., HEGNSVAD, D. et al. Mechanical properties of the human Achilles tendon, in vivo. **Clinical Biomechanics**, v. 25, n. 7, 2011.

LIU, M.Q., ANDERSON, F.C., SCHWARTS, M.H. Muscle contributions to support and progression over a range of walking speeds. **Journal of Biomechanics**, v. 41, n.15, p.3243-3253, 2008.

LONGO, U.G., RONGA, M., MAFFULLI, N. Achilles tendinopathy. **Sports Medicine and Arthroscopy Review**, v.17, n.2, p.112-126, 2009.

LUND, H,. SONDERGAARD, K., ZACHARIASSEN, T. et al. Learning effect of 12 isokinetic measurements in healthy subjects, and reliability and comparability of 13 Biodex and Lido dynamometers. **Clinical Physiology and Functional Imaging**, v. 25, n. 14 p.75-82, 2005.

MAFFULLI N., WALLEY, G., SAYANA, M.K, et al. Eccentric calf muscle training in athletic patients with Achilles tendinopathy. **Disability and Rehabilitation**. v.30, n.20, p.1677-1684, 2008.

MORRIS-CHATA, R., BUCHNER, D.M, DE LATEUR, B.L. et al. Isokinetic testing of ankle strength in older adults: assessment of inter-rater reliability and stability of strength over six months. **Archives of Physical Medicine and Rehabilitation**, v. 75, n.11, p. 1213-1216, 1994.

OBERG, B., BERGMAN, T., TROPP, H. Testing of isokinetic muscle strength in the ankle. **Medicine and Science in Sports and Exercise**, v.19, n.7,p. 318–322, 1987.

PORTNEY, L.E, WHATKINS, M.P. **Foundations of Clinical Research: Applications and Practice,** 3rd edition. Prentice Hall, New Jersey, 2009.

SCHWENK, M., GOGULLA, S., ENGLERT, S., CZEMPIK, A., HAUER, K. Test-retest reliability and minimal detectable change of repeated sit-to-stand analysis using one body fixed sensor in geriatric patients. **Physiological Measurement**, v.33, n.11, p.1931-1946, 2012.

STRATFORD, P. W., BRUULEMA, A., MAXWELL, B. et al. The effect of inter-trial rest interval on the assessment of isokinetic thigh muscle torque. **The Journal of Orthopaedic and Sports Physical Therapy**, v. 11, n.8, p. 362–366, 1990.

WENNERBERG., D. Reliability of an isokinetic dorsiflexion and plantar flexion apparatus. **The American Journal of Sports Medicine**, v.19, n.5, p. *19:* 519–522, 1991.

5 CONCLUSION

The literature review presented in the present work had its main focus on basic concepts about the anatomical aspects of the Achilles tendon and the plantar flexor muscles. Methods for evaluating the mechanical and morphological properties of the triceps surae in healthy subjects were presented. However, controversial results were found in the literature considering the modifications promoted in these properties in healthy individuals. In addition, the studies showed methodological deficiencies in the form of evaluation of both mechanical and morphological properties. Thus, there was a gap in the literature regarding the studies that evaluated the triceps surae psychometric properties. These aspects served as motivation for the development of the subsequent three cross-sectional studies that make up the present master's dissertation.

The first study showed a high reliability observed for all muscle architecture parameters (fascicle length, pennation angle and muscle thickness) of the plantar flexor muscles, between inter-analyzers, inter-raters and intra-raiser. These results demonstrated that our muscle parameters measurements are reliable for evaluating the muscular architecture when they are performed by the same evaluator at different times, and when performed by different evaluators and analysts.

The second cross-section study showed a high reliability for the Achilles tendon length and CSA measurements for the between-evaluators comparisons and among the analysts, showing that these measures are also reliable in the evaluation when performed by different evaluators and analysts. In contrast, the low reproducibility found for free tendon length in the between-evaluators comparisons suggests that these parameters are dependent on the evaluator, that a longer period of training before data collection should be performed and that this measure should be performed with caution with patients post Achilles tendon rupture until we have an adequate precision of the measure.

The third study showed a high reliability for all intra- and inter-rater tests, indicating that these measures are reliable when evaluating the triceps surae strength production capacity by the same evaluator at different times, and when performed by different raters. In addition, the strength assessment results allow us to obtain reliable mechanical properties of the triceps surae in healthy individuals, which can be used as a good and reliable strength measurement for comparison with Achilles tendon patients in clinical practice.

Finally, from these results it is clear that the systematized evaluation proposed and tested in the present study may have an impact on clinical decision making. However, improvements in the used techniques are necessary in order to improve reliability and further reduce measurement errors at those specific points where reliability was considered low in the evaluation of triceps surae mechanical and morphological parameters.