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ENSAIOS BIOMECÂNICOS DO TENDÃO CALCÂNEO DE RATOS POR MEIO DE MÁQUINA MULTIAXIAL

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Trabalho de Conclusão de Curso apresentado à Universidade de Brasília – UnB – Faculdade de Ceilândia como requisito parcial para obtenção do título de bacharel em Fisioterapia.
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Dedicatória

Dedico este trabalho a minha família que não mediu esforços para me dar a educação desejada e considerada por eles a melhor, a todos que me apoiaram em todos os momentos que precisei e à comunidade acadêmica.

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À minha orientadora Rita Marqueti por todo apoio, preocupação e ensinamentos durante quase toda a graduação. Obrigado por confiar em mim e acreditar que eu tenho potencial em tornar-me um pesquisador.

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Epígrafe

"Não existe pedra no seu caminho que você não possa aproveitá-la para o seu crescimento." (Autor desconhecido)

RESUMO

OLIVEIRA, Anderson José Santana. Ensaios biomecânicos do tendão calcâneo de ratos por meio de máquina multiaxial. 2016. 39f. Monografia (Graduação) - Universidade de Brasília, Graduação em Fisioterapia, Faculdade de Ceilândia. Brasília, 2016.

Referencial teórico: O tendão é um componente anatômico composto por tecido conjuntivo denso modelado composto principalmente por colágeno I, composto relacionado diretamente com a função mecânica de transmitir e suportar cargas. A função tendínea pode ser avaliada a partir do aumento do estresse mecânico deste tecido. Porém, a literatura ainda é escassa com relação a padronização de ensaios biomecânicos para o tendão calcâneo. Este estudo teve o objetivo de padronizar ensaios de tração de tendões calcâneos de ratos em uma máquina de ensaio multiaxial a partir do desenvolvimento e confecção de um par de mordentes e analisar a função do tendão por meio de variáveis biomecânicas.
Métodos: Foram utilizados quatro tendões calcâneos de ratos sadios, machos, com idade de 10 semanas (n = 2). Os animais foram eutanasiados utilizando-se injeção intraperitoneal de solução de xilazina e quetamina para retirada dos tendões, os quais foram presos por mordentes, interconectados a uma célula de carga e a uma máquina de ensaio multiaxial. Os tendões foram submetidos a um aumento gradual de carga a uma taxa de deslocamento constante de 1mm/min. O projeto de pesquisa foi aprovado pelo Comitê de Ética em Experimentação Animal da Universidade Católica de Brasília (protocolo 028/15).
Achados: Obteve-se curvas de força-deslocamento, demonstrando a efetividade do ensaio. Foi necessária uma força de 26,08N para romper o tendão, com comprimento aumentado em 2,08mm, correspondente a 23,58% do seu comprimento. Apresentou capacidade máxima de suportar uma tensão de 28,55MPa. A energia suportada pelo tendão até o momento de sua ruptura foi de 21,78J.
Conclusão: O presente estudo obteve sucesso devido a efetividade de mensurar a força, o deslocamento, a tensão e a deformação máxima do tendão. Porém, sugere-se novos estudos com maior número de amostras.

Palavras-chave: Tendão calcâneo, resistência à tração, estresse mecânico.

ABSTRACT

OLIVEIRA, Anderson José Santana. Biomechanical tests of the Achilles tendon of rats by multiaxial machine. 2016. 39f. Monograph (Graduation) - University of Brasilia, Undergraduate Course of Physiotherapy, Faculty of Ceilândia. Brasília, 2016.

Background: Tendon is anatomic component compost of dense connective tissue modeling, which is mainly compost of collagen type I, and it is directly related to mechanical function of transmit and support loads. Tendon function may be availed for increasing mechanical stress of its. However, the literature is scarce in relation to the standardization of mechanical tests of the calcaneus tendon. This aim of this study was to standardize mechanical tests of calcaneus tendon of healthy rats by multiaxial test machine by developing and making a pair of clamps, and analyze tendon function by mechanical variables.
Methods: We used four Achilles tendons of healthy, male, ten-years-old rats (n = 2). The animals were euthanized giving an injection of xylazine and ketamine solution in rats' intraperitoneal region to withdraw the tendons, whose tendons were clamped, connected to a cell of charge and then to a multiaxial test machine. The tendons were submitted to a gradual increase of length at a constant rate of 1mm/min. The experimental procedures were supported by Ethics Committee in Animal Research of Catholic University of Brasília (protocol number 028/2015).
Findings: We obtained force-displacement curve, which it showed the effectivity of the test. It was necessary a force of 26,08N to the tendon rupture, with extension of 2,08mm, what it corresponds to 23,58% of its length. It supported a maximal tension of 28,55MPa. The energy supported by tendon until its rupture was 21,78J.
Interpretation: This study was successful because it got to measure the maximum force, extension, stress and strain of tendon. However, we suggest new studies using a larger sample.

Keywords: Calcaneus tendon, tensile strength, mechanical stress.

SUMÁRIO

LISTA DE ABREVIATURAS

CP – Corpos de prova

GAGs – Glicosaminoglicanos

GMEC – Grupo de Mecânica Experimental e Computacional

kg – Quilograma

kgf – Quilograma força

kN – Quilonewton

MEC – Matriz extracelular

MPa – Megapascal

mg – Miligrama

min – Minuto

mm – Milímetro

PGs – Proteoglicanos

TC – Tendão calcâneo

LISTA DE TABELAS E FIGURAS

1. INTRODUÇÃO

O tendão é um componente anatômico dos músculos estriados esqueléticos constituído de tecido conjuntivo denso modelado que fixam os músculos ao esqueleto com finalidade altamente específica de transmitir força da contração muscular a unidade óssea de inserção (Fang and Lake, 2016; Kannus, 2000; Kjaer, 2004; Lacroix et al., 2013; Screen et al., 2015), demonstrando assim grande importância biomecânica na geração de movimentos e estabilização das articulações (Joseph et al., 2014; Kannus, 2000; Screen et al., 2015; Wang, 2006).

Morfologicamente, o tendão pode ser definido como um conjunto de feixes inelásticos de colágenos organizados hierarquicamente (Figura 1), composto por um conjunto de fascículos, fibras, fibrilas e moléculas de colágeno (Fang and Lake, 2016; Freedman et al., 2014; Nourissat et al., 2015; Screen et al., 2015; Wang, 2006).

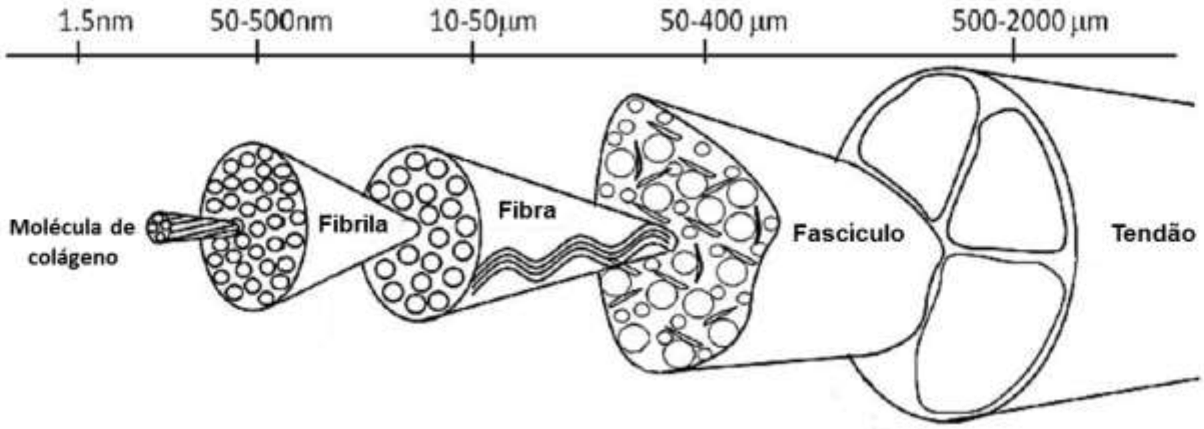

Figura 1: *Organização hierárquica do tendão* (adaptado de Screen et al., 2015).

Sua matriz extracelular (MEC) é composta basicamente de água e colágeno, sendo o colágeno tipo I o mais abundante, o que torna o tendão tão resistente a cargas de tensão (Aquino et al., 2005; Freedman et al., 2014; Kannus, 2000; Kjaer, 2004; O'Brien, 1997). Porém, ainda encontram-se colágeno tipo III, IV, V e VI, PGs, glicosaminoglicanos (GAGs), glicoproteínas estruturais e substâncias inorgânicas (Kjaer, 2004; Yoon and Halper, 2005).

Estruturalmente, e diferentemente do que se pensa, as fibras de colágeno no seu estado de repouso, isto é, com ausência de forças sobre elas, não se apresentam em estado tenso, e sim em um aspecto ondulado, denominado "*crimp*" (Figura 2) (Maganaris et al., 2008; Wang, 2006). Assim, este aspecto é correspondente à função viscoelástica do tendão, a qual pode ser avaliada a partir de um teste biomecânico ou teste de tração tendínea (Aquino et al., 2005; Maganaris et al., 2008), que tem a principal finalidade de identificar de forma quantitativa a força que o tecido pode suportar conforme seu alongamento, gerando uma curva força-deslocamento, da qual deriva-se uma curva tensão-deformação (Young et al., 2016).

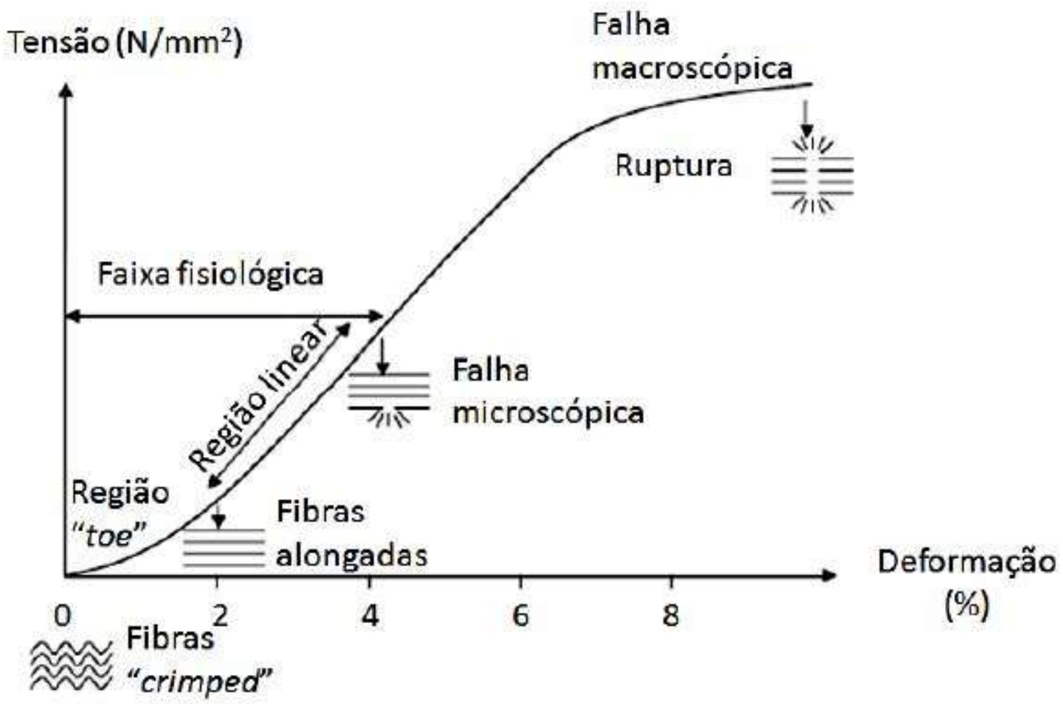

Figura 2: *Curva tensão-deformação (stress-strain) (adaptado de Wang, 2006).*

A curva tensão-deformação (stress-strain) pode ser dividida em quatro regiões: a região "*toe*", caracterizada pelo alinhamento das fibras de colágeno, saindo do estado "*crimped*", mas sem promover o alongamento das fibras; a segunda região está relacionada ao alongamento das fibras de colágeno de forma elástica, sem causar danos ao tecido, caracterizada no gráfico como uma região linear; a terceira e quarta região são correspondentes a fase plástica do tecido, momento que as microlesões das fibras de colágeno começam a ocorrer, até seu rompimento completo, respectivamente (Figura 2) (Aquino et al., 2005; James et al., 2008; Maganaris et al., 2008; Wang, 2006). Contudo, extraem-se dados a respeito da elasticidade, correspondente à faixa fisiológica, plasticidade, rigidez, ponto de falha e energia absorvida até a ruptura tecidual (Aquino et al., 2005).

Além desses parâmetros, extraem-se ainda o módulo de elasticidade ou módulo de Young e a energia suportada pelo tecido (James et al., 2008; Marqueti et al., 2011; Nakagaki et al., 2007; Wang, 2006).

A função tendínea de suportar e transmitir cargas está diretamente relacionada à composição de sua MEC (Freedman et al., 2014; Screen et al., 2015). Apesar disso, afirma-se que a análise das propriedades biomecânicas quando comparada à morfológica, mostra-se como a melhor maneira de se avaliar a função tendínea (Bohm et al., 2015).

Todavia, mesmo com sua relevância para analisar a função tendínea, a literatura apresenta-se escassa em relação a estudos com análise biomecânica de TC de ratos, variando entre estudos de imagens em diversos tendões de humanos (Heinemeier and Kjaer, 2011; Kubo et al., 2003; Maganaris et al., 2004; Pierre-Jerome et al., 2010) e de outros animais (Dourte et al., 2013; Dudhia et al., 2007; Marqueti et al., 2011). Com isso, há necessidade de novos estudos visto a importância de se verificar a função de tendões.

Portanto, o objetivo deste estudo foi padronizar ensaios de tração de tendões calcâneos de ratos em uma máquina de ensaio multiaxial a partir do desenvolvimento e confecção de um par de mordentes, os quais permitirão analisar a função do tendão calcâneo por meio das variáveis força, deslocamento, tensão, deformação e energia máxima suportada pelo tecido. Além disso, analisar graficamente as regiões elástica e plástica do TC.

2. MÉTODOS

Foram utilizados quatro tendões calcâneos de ratos Wistar *novergicus albinus* sadios, machos, com peso médio de 314g, com idade de 10 semanas (n = 2). Os animais foram eutanasiados utilizando-se injeção intraperitoneal de xilazina e quetamina (24mg/kg de peso corporal e 190mg/kg de peso corporal, respectivamente) para a retirada dos tendões e realização do experimento. Assim que extraídos, os tendões foram conservados em solução salina à 4°C até o momento dos ensaios biomecânicos, no máximo 72 horas após a retirada.

Um par de mordentes para segurar os corpos de prova (CP) foi confeccionado, com largura de 10mm, comprimento de 20mm e espessura de 5mm, cada. Além disso, foram confeccionados conectores para interligar os mordentes a célula de carga de 0,2kN (S2M, 200N, HBM) e à máquina, a qual foi necessária para sensibilizar a aquisição de dados do ensaio (Figura 3A). Foi utilizada uma lixa metálica número 80, a qual foi colada na face interna de cada mordente para uma maior aderência ao tecido tendíneo, evitando que os tendões deslizassem dos mesmos durante o teste.

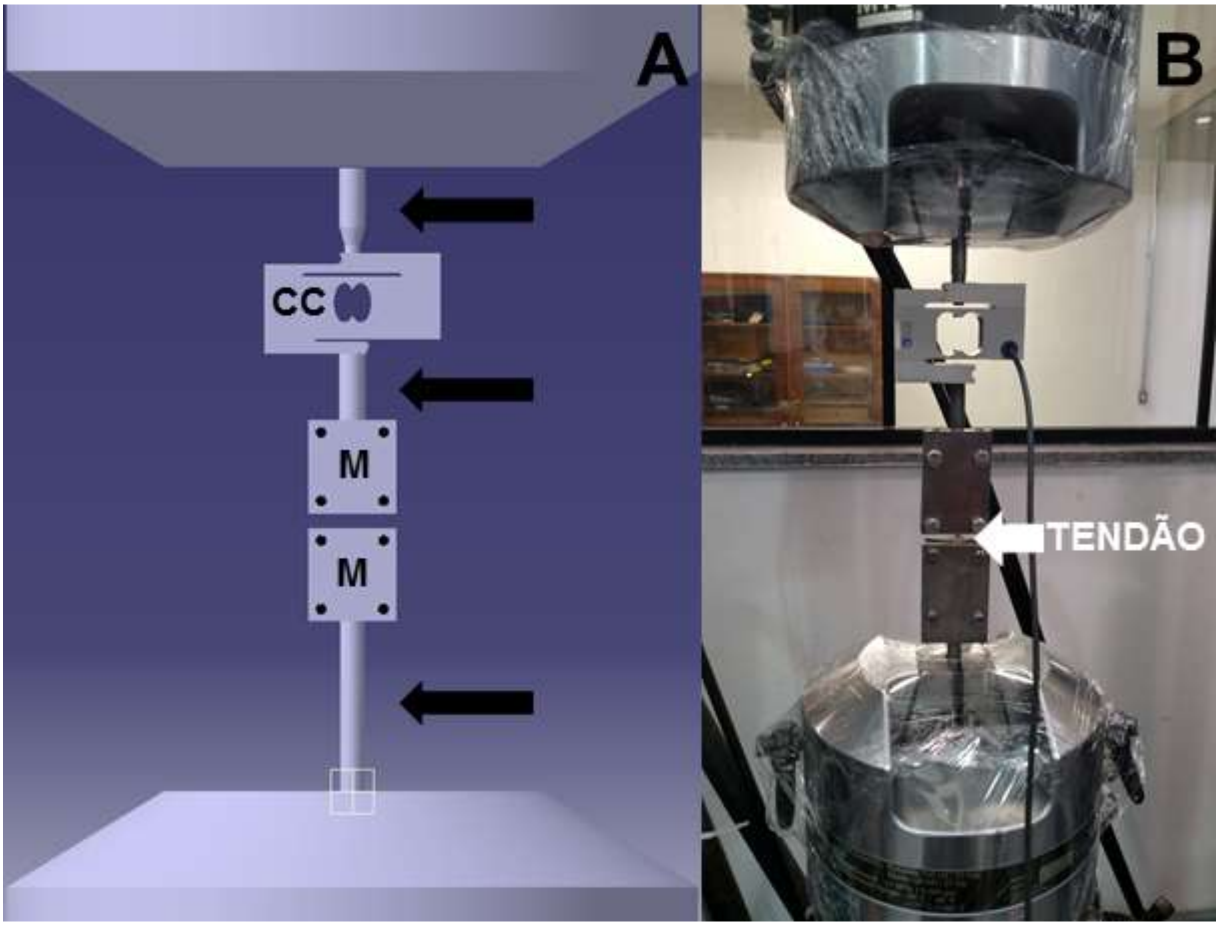

Figura 3: *A, modelo de ensaio biomecânico previamente idealizado; B, realização do teste biomecânico do tendão calcâneo. Setas pretas: conectores; M: mordente; CC: célula de carga de 0,2kN.*

Os ensaios biomecânicos ocorreram a partir de uma máquina de ensaios universal multiaxial (MTS Landmark 370.10) disponível no Laboratório de Ensaios de Materiais do Departamento de Engenharia Mecânica da Universidade de Brasília.

A máquina foi revestida de papel filme para evitar contato do material biológico com finalidade de prevenir danos ao instrumento do laboratório e mantê-la higienizada. Instantaneamente, os CP foram presos aos mordentes, e foi utilizado um paquímetro digital (modelo CD-6"CSX-B, série Absolute, Mitutoyo) parar mensurar o comprimento, a largura e a espessura dos tendões, submetendo-os a um aumento gradual de carga a uma taxa de deslocamento constante de 1mm/min (Figura 3B).

O experimento foi conduzido seguindo as recomendações éticas do Conselho Nacional de Experimentação Animal (COBEA). O projeto de pesquisa foi aprovado pelo Comitê de Ética em Experimentação Animal da Universidade Católica de Brasília (protocolo nº 028/15).

Realizado o ensaio biomecânico, adquiriu-se uma curva força-deslocamento (N/mm), a uma frequência de 5Hz (Figura 4A), a qual foi filtrada para frequências menores por meio do software MATLAB® (The MathWorks, Inc), para extrair um resultado mais fidedigno (Figura 4B, C e D).

Após a filtragem de sinais, verificou-se que o último filtro, com menor frequência, diferenciava-se dos anteriores e do sinal original, apresentando diminuição dos valores durante toda onda (Figura 4D). Em vista disto, escolheu-se o sinal com frequência de 0,15Hz (Figura 4C) para extrair os resultados do ensaio biomecânico. Os resultados foram extraídos a partir do início do teste até o ponto de ruptura do TC (Figura 5).

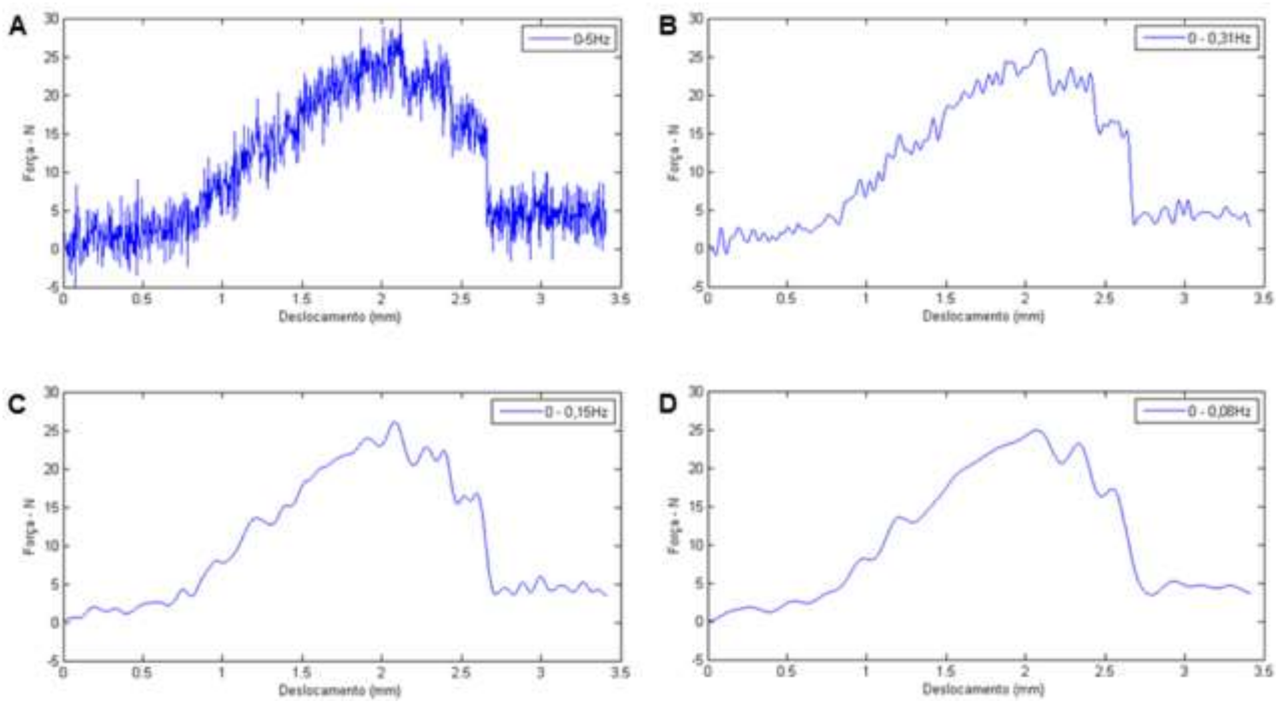

Figura 4: *Curvas de Força (N) - Deslocamento (mm) extraídas a partir do ensaio de tração do tendão calcâneo. A: sinal original com frequência de 5Hz; B: sinal filtrado com frequência de 0,31Hz; C: sinal filtrado com frequência de 0,15Hz; D: sinal filtrado com frequência de 0,08Hz. Os sinais B, C e D foram filtrados a partir do sinal original.*

O gráfico tensão-deformação foi adquirido a partir do gráfico força deslocamento, sabendo que a tensão gerada no tecido (MPa) é igual a força imposta a ele (N), dividido pela área de secção transversa do TC (mm^2); e que a deformação do tecido é igual ao deslocamento do tecido no ensaio (mm), dividido pelo comprimento do tecido (mm), multiplicado por 100.

Além disso, para verificar a energia (E) suportada pelo tendão foi necessário calcular a área abaixo do gráfico até o momento de sua ruptura, expressa pela seguinte equação (Wu et al., 2004):

$$E = (y_1 + y_2) * \frac{\Delta x}{2} + (y_2 + y_3) * \frac{\Delta x}{2} + (y_3 + y_4) * \frac{\Delta x}{2} + \cdots + (y_{698} + y_{699}) * \frac{\Delta x}{2} + (y_{699} + y_{700}) * \frac{\Delta x}{2}.$$

Simplificando-a, tem-se:

$$E = \Delta x * \frac{(y_1 + y_{700})}{2} + \sum y - y_1 - y_{700}$$

Em vista que y é considerado ao valor de força adquirida, dentre os 700 pontos presentes no gráfico (Figura 5A), e Δx corresponde à variação de deslocamento entre o ponto "y" inicial e final. Todavia, como a aquisição dos dados foi em função do deslocamento por tempo (1mm/min), tem-se a taxa de deslocamento contínua, ou seja:

$$\Delta x = 0{,}00333$$

3. RESULTADOS

Foram realizados quatro ensaios, sendo destes um efetivo, ou seja, em somente um ensaio não houve o deslizamento do CP, seja da porção muscular ou da pata. O deslizamento destas porções era caracterizado pelo aumento do deslocamento exercido pela máquina, porém, não se identificava o aumento progressivo da força no tecido. Assim, verificou-se que foi necessária uma força de 26,08N ou 2,66kgf para romper o tendão, o qual teve seu comprimento aumentado em 2,08mm (Figura 5A, Tabela 1).

Tabela 1: *Valores das propriedades estruturais e mecânicas do tendão calcâneo utilizado no ensaio biomecânico.*

	Medidas
Propriedades Estruturais	
Comprimento inicial (mm)	8,82
Área de secção transversa (mm²)	0,91
Propriedades Mecânicas	
Força máxima (N)	26,08
Deslocamento máximo (mm)	2,08
Tensão máxima (MPa)	28,55
Deformação máxima (%)	23,58
Energia máxima (J)	21,78

Observou-se que o tendão apresentou capacidade máxima de suportar uma tensão de 28,55MPa, obtida a partir da divisão da força máxima para romper o tendão sobre a área de secção transversa do tendão, originada pela multiplicação da espessura e da largura do TC.

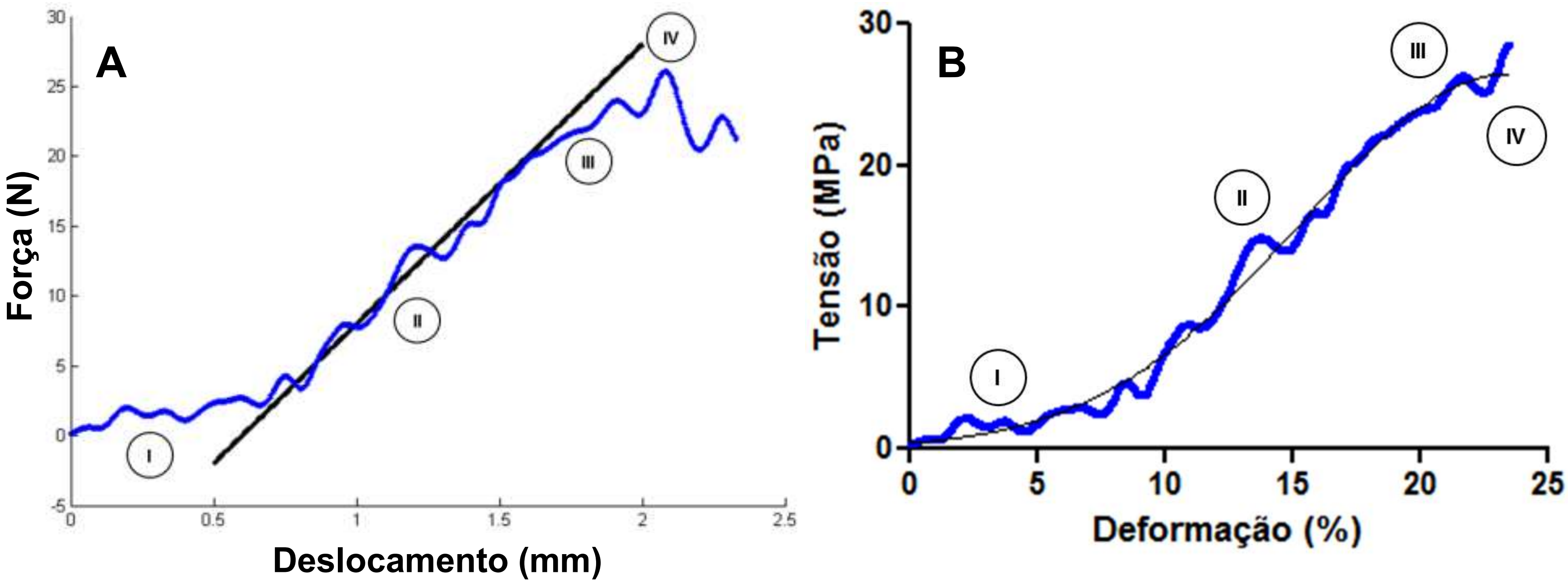

Figura 5: *A, curva força (N) – deslocamento (mm) do tendão calcâneo com frequência de 0,15Hz, com a região linear da curva evidenciada; B, curva tensão (MPa) – deformação (%) do tendão calcâneo com curva de tendência. I: região toe; II: região linear; III: início da fase plástica do tecido; IV: ponto de ruptura total do tecido.*

Além disso, o tendão apresentou deformação equivalente a 23,58% do seu comprimento inicial (Figura 5B, Tabela 1). Com isso, obteve-se o valor de 21,78J, sendo este o valor máximo de energia absorvida pelo TC.

Outro fator observado foi uma região similar a uma reta, a qual é referente à fase linear de tração do tendão, correspondente à fase elástica do tecido (Figura 5A, II). A fase plástica do tecido iniciou a partir do instante em que a curva se distanciou da reta (Figura 5A, III), fase caracterizada pela ruptura parcial das fibras de colágeno.

4. DISCUSSÃO

Em vista que o objetivo primário era padronizar o ensaio de tração do tendão, afirma-se que tal meta foi cumprida. Verificou-se que para o TC romper foi necessária uma força de aproximadamente 26,1N, corroborando com o grupo controle de um estudo que avaliou diferentes modelos de imobilização da pata traseira de ratos (Min et al., 2013), no qual foi necessária uma força de 29,33±3,38N para que houvesse a descontinuidade do tecido (Min et al., 2013). Diferentemente, Tsitsilonis et al. (2014) mensuraram uma média de força maior que os presente e o estudo citado.

Em contrapartida ao resultado semelhante, a energia absorvida pelo TC até sua ruptura foi menor comparado a outros estudos (Min et al., 2013).

Outro estudo, encontrou que a tensão máxima suportada pelo TC do grupo controle foi de 28,5±4,3MPa, semelhante ao presente estudo, e que os tendões deste grupo apresentaram uma deformação de 21,1±1,7%, próximo ao valor encontrado de 23,58% (Heinemeier and Kjaer, 2011).

Portanto, o presente estudo mostrou-se relevante para avaliar a função do TC com possibilidade de dar suporte a novos estudos que almejem avaliar a função de tendões, expostos ou não a diferentes condições, como sexo e idades diferentes. O teste biomecânico é encarado como o principal teste para avaliar a função tendínea (Bohm et al., 2015), que se mostra relevante para a área da fisioterapia devido a análise de como seria o comportamento biomecânico de tecidos moles frente ao estresse máximo, e como este tecido se comporta elasticamente.

Com isso, a análise biomecânica deste projeto, realizada por meio de ensaio de tração, pode investigar distintas propriedades dentre elas força, deslocamento, tensão, deformação e energia máxima que o este tecido suportou até sua ruptura total, propriedades que clinicamente podem dar base a reflexões a respeito da realização de cirurgia (Huang et al., 2015), pré e pós-cirúrgico, e recuperação ou prevenção de rupturas (Huang et al., 2015; Zhang et al., 2016).

Dentre as limitações do presente trabalho, podemos descrever que diferentemente do que se encontra na maioria dos estudos, os gráficos apresentados demonstram ruídos durante toda a curva, o qual pode ser associado à não utilização da célula de carga, equivalente a 0,2kN, devido à inexistência do aquisitor de dados no momento da coleta, sendo utilizado então a célula de carga da própria máquina de ensaio. A célula de carga da máquina é correspondente a 0,5kN, a qual não é adequada para testar materiais biológicos, como os tendões. Para a avaliação dos tecidos biológicos é recomendado utilizar uma célula de carga com capacidade similar à transmissão da força do tecido alvo. Sendo assim, a célula de carga deve funcionar como um transdutor de força e ser capaz de transformar uma grandeza física (força em um sinal elétrico). Quanto mais específica for a célula de carga, mais fidedigno será o sinal transmitido e melhor será a aquisição das alterações elásticas e plásticas no tecido. A maioria dos equipamentos destinados à engenharia de materiais além de robustos são otimizados para realizar testes em materiais diferentes dos biológicos, como concreto, aço, ferro, cerâmica entre outros. E por isso, suas células de carga, geralmente já acopladas aos equipamentos são adequadas para testes nesses tipos de materiais.

Todavia, tem-se a perspectiva de adaptar o estudo realizado a outro modelo de máquina de ensaios mecânicos para a realização de futuros estudos. Além disso, já está em desenvolvimento a confecção de um novo modelo de mordentes com o intuito de otimizar o tempo gasto para iniciar o teste, pois em comparação à duração dos testes biomecânicos, gastava-se mais tempo no alinhamento e preparação dos tendões para a realização do teste do que o teste propriamente dito, o que pode beneficiar outros estudos sejam na utilização de diferentes tendões ou outros tipos de tecidos.

5. CONCLUSÕES

Conclui-se que o presente estudo, por meio de um ensaio biomecânico até o ponto de ruptura total do tendão calcâneo de rato, foi capaz demensurar a força, o deslocamento, a tensão, a deformação e a energia máxima suportada pelo tendão calcâneo importantes para padronização de ensaios futuros. Entretanto, necessita-se repetir o protocolo com maior número de amostras para uma maior confiabilidade.

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7. ANEXOS

ANEXO A – NORMAS DA REVISTA

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A journal affiliated to the International Society of Biomechanics, the American Society of Biomechanics, the European Society of Biomechanics and the Taiwanese Society for Biomechanics

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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ANEXO B – PARECER DO COMITÊ DE ÉTICA

CEUA Universidade Católica de Brasília

CERTIFICADO

Certificamos que o projeto intitulado "**Lesões músculo-tendíneas e sua relação com a regeneração tecidual e remodelamento da matriz extracelular**", protocolo nº **028/15** sob a responsabilidade de Rita de Cássia Marqueti Durigan - que envolve a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA/UCB) DA Universidade Católica de Brasília, em reunião de 18 / 11 / 2015.

Vigência do projeto	30 de julho de 2019.
Espécie / linhagem	*Rattus norvergicus* / Wistar
Nº de Animais	140
Peso / Idade	200g / 2 Meses
Sexo	Masculino
Origem	Biotério do Laboratório de Processos Básicos (UCB)

Brasília, 18 de Novembro de 2015.

Secretaria da Comissão de Ética
Em Uso de Animais (CEUA/UCB)
Universidade Católica de Brasília
(61) 3356-9492

Tara maria B. Rezende