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NEW PERSPECTIVE IN A CROSS- SECTIONAL STUDY ON MUSCLES AND TENDONS IMPAIRMENTS DUE TO NEUROMUSCULAR MALADAPTED FUNTION FOUND IN CHRONIC SPINAL CORD INJURY

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BRASÍLIA - DF
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Tese de mestrado apresentada ao Programa de Pós-graduação em Ciências da Reabilitação da Universidade de Brasília – UnB como requisito parcial para obtenção do título de mestre em Ciências da Reabilitação.

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DEDICATÓRIA

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SUMÁRIO

RELAÇÃO DE FIGURAS E TABELAS

RELAÇÃO DE ABREVIATURAS

LM: Lesão Medular

SET: Eletrodiagnóstico por Estímulo

NMES: Estimulação Elétrica NeuroMuscular

NED: Distúrbios Eletrofisiológicos Neuromusculares

ASIA: do inglês, *American Spinal Injury Association*

BB: Bíceps Braquial

RF: Reto Femoral

VL: Vasto Lateral

VM: Vasto Medial

TA: Tibial Anterior

TQ: Tendão Quadriceps

TP: Tendão Patelar

GP: Grupo Pareado

GLM: Grupo Lesão Medular

ICC: Coeficiente de correlação

CSA: Área de secção transversa

AI: Índice de acomodação (accommodation/rheobase)

RESUMO

Introdução: Os comprometimentos da lesão medular (LM) resultam em distúrbios do movimento quase sempre manifestados por músculos paralisados com inervações preservadas e suscetíveis de serem ativados por estimulação elétrica. **Objetivo:** Avaliar alterações e associações na função neuromuscular e na estrutura musculoesquelética, nos músculos paralisados com inervações preservadas, em pessoas com LM, comparado a um grupo pareado hígido (GP). **Métodos:** Foi realizado um estudo de caso-controle transversal para comparar músculos saudáveis e comprometidos (bíceps braquial [BB], reto femoral [RF], vasto lateral [VL], vasto medial [VM] e tibial anterior [TA]) e tendões (quadríceps e patelar) por meio de parâmetros obtidos no teste eletrodiagnóstico de estímulo (cronaxia, reobase, acomodação e índice de acomodação) e avaliação por ultrassonografia (espessura, ângulo de penação, comprimento do fascículo e ecointensidade). Ao mesmo tempo, foram realizadas medidas repetidas de dois avaliadores independentes para verificar a confiabilidade entre as medidas. **Resultados:** Trinta participantes (quinze em cada grupo) foram avaliados. Em relação aos parâmetros da ultrassonografia, foram encontrados interação músculo × grupo estatisticamente significante: espessura muscular ($F = 2,96$, $p<0,022$, ICC 0,91), ecointensidade ($F = 6,74$, $p<0,0001$, ICC 0,90), ângulo de penação ($F = 5,52$, $p<0,0001$, ICC 0,89) e comprimento fascicular ($F = 2,71$, $p<0,0497$). Na arquitetura dos tendões, houve diferença estatisticamente significante na espessura do TQ quando comparado ao GP ($p<0,046$, ICC 0,94). Em relação aos valores de cronaxia, foram demonstradas diferenças significativas entre músculo × grupo ($F = 15,58$, $p <0,0003$, ICC 0,87). O grupo LM apresentou prevalência de distúrbios eletrofisiológicos neuromusculares (NED) de 33,3% nos músculos RF e VL e 26,6% nos músculos VM e TA. **Conclusão:** A atrofia por desuso ocorre globalmente em pacientes com LM crônica abaixo do nível da lesão, reduzindo a espessura muscular, o ângulo de flexão e o comprimento fascicular, além de aumentar a ecogenicidade dos músculos e tendões. O aumento dos valores da cronaxia e de NED parece desempenhar um papel fundamental na má adaptação da arquitetura musculotendínea.

ABSTRACT

Introduction: Spinal cord injury (SCI) impairments result in movement disorders manifested by paralyzed muscles, with preserved innervations susceptible to activation by electrical stimulation. **Objective:** To evaluate changes and associations in neuromuscular function and musculoskeletal structure in paralyzed muscles with preserved innervations in people with SCI in comparison with able-bodied people. **Methods:** A cross-sectional case-control study was designed to compare healthy and impaired muscles (biceps brachii [BB], rectus femoris [RF], vastus lateralis [VL], vastus medialis [VM], and anterior tibialis [TA]) and tendons (quadriceps and patellar) obtained from the stimulus electrodiagnostic testing (chronaxie, rheobase, accommodation, and accommodation index) and ultrasound assessment (thickness, pennation angle, fascicle length, and eco intensity). At the same time, repeated measures from two independent raters to check the inter-rater reliability of the measures were performed. **Results:** Thirty participants (fifteen in each group) were evaluated. Regarding US parameters, a significant muscles *versus* impairment interaction was found in the muscle thickness ($F = 2.96$, $p < 0.022$, ICC 0.91), echo intensity ($F = 6.74$, $p < 0.0001$, ICC 0.90), pennation angle ($F = 5.52$, $p < 0.0001$, ICC 0.89), and fascicle length ($F = 2.71$, $p < 0.0497$). In tendon architecture, we found quadriceps tendon thickness reduction comparing SCI and able-bodied individuals ($p < 0.046$, ICC 0.94). Regarding chronaxie values, muscles *versus* impairment ($F = 15.58$, $p < 0.0003$, ICC 0.87) revealed relevant differences. In addition, the SCI had a higher prevalence of Neuromuscular Electrophysiological Disorders (NED) of 33.3% in the RF and VL, and 26.6% in the VM and TA muscles. **Conclusion:** Disuse atrophy occurs globally in individuals with chronic SCI for the muscles under the lesion level, reducing muscle thickness, pennation angle, and fascicular length, as well as increasing muscle and tendon echogenicity. The increased chronaxie values and higher NED prevalence for SCI seem to play a key-role in the maladaptation of musculotendinous architecture, revealing a possible acquired peripheral neuropathy.

1. INTRODUÇÃO

1.1. Lesão Medular e atrofia muscular por desuso

A lesão medular (LM) é considerada mundialmente um problema de Saúde Pública (REF). Segundo a Associação Americana de Lesão Medular (do inglês, *American Spinal Injury Association* - ASIA), a LM define-se pela diminuição ou ausência de função motora e/ou sensória e/ou anatômica, caracterizando-se como lesão completa ou incompleta em decorrência do comprometimento de elementos neuronais presentes no interior do canal vertebral (Kirshblum et al., 2011). As deficiências da LM resultam em distúrbios do movimento quase sempre manifestados por músculos paralisados com inervações preservadas e, portanto, suscetíveis de serem ativados por estimulação elétrica (Harvey, 2016; Rabelo et al., 2017).

Abaixo do nível da lesão, a perda somestésica, proprioceptiva, do controle neuromotor e de funções autonômicas acarreta alterações profundas como hipoestesia/anestesia, paresia/paralisia e disfunção visceral, vasomotora, esfincteriana e sexual (McDonald e Sadowsky, 2002; Van Middendorp el al., 2011). Em relação à categoria neurológica, no momento da alta hospitalar, a maioria dos pacientes apresenta tetraplegia incompleta (41,2%), seguida pela paraplegia incompleta (19,6%), tetraplegia paraplegia completa (20,2%) e tetraplegia completa (12,3%) (National spinal cord injury Statistical Center, 2020). Diante disso, a LM também afeta o bem-estar físico, social e psicológico das vítimas e gera uma demanda substancial aos sistemas de saúde, famílias e comunidades (Singh et al., 2014).

A incidência anual de LM vai de 12, na Espanha, a 54 casos por milhão de pessoas nos Estados Unidos (National spinal cord injury Statistical Center, 2020). A maior parte dos estudos mostra uma razão alta de homens para mulheres, idade de pico de incidência abaixo de 30 anos. Acidentes automobilísticos são as principais causas de lesão, seguida por quedas. Atos violência (principalmente ferimentos a bala) e atividades esportivas / recreativas também são relativamente causas comuns (National spinal cord injury Statistical Center, 2020). Dados no Brasil são escassos, mas estima-se que ocorram 10.000 novos casos por ano (Ministério da Saúde, 2015) com características semelhantes às de outros países, a exemplo dos acidentes automobilísticos, que também são a principal etiologia no município de Aracaju/SE (40,8%). Porém, no mesmo local, a violência como causa da LM é mais expressiva que internacionalmente, estando em torno de 32,5%,

somando ferimento por arma de fogo e por arma branca e agressão física (McDonald e Sadowsky, 2002; Singh et al., 2014).

Os avanços em cuidados após a lesão e durante a recuperação levaram a um aumento na taxa de sobrevivência e na expectativa de vida dos indivíduos com LM (Middleton et al., 2012). Contudo, diversas complicações são observadas, e os prejuízos resultantes da lesão na medular variam dependendo da organização funcional da medula espinhal e o local da lesão. A perda de função muscular leva a limitações no desempenho de atividades funcionais como a marcha, a habilidade de transferir-se e manter-se na posição sentada (Van Middendorp et al., 2011).

O efeito mais visível da paralisia total ou parcial da musculatura é a atrofia muscular, caracterizada por diminuição do tamanho das fibras musculares, associada a uma complexa cascata de efeitos bioquímicos que afetam a composição do músculo atingido. Funcionalmente, essas mudanças manifestam-se através da perda de força, de potência e de resistência muscular (Castro et al., 1999, Dumitru et al., 2017). A atrofia de um músculo pode ocorrer principalmente de duas maneiras, devido ao desuso ou desnervação, e uma variedade de patologias desencadeiam uma reação à atrofia por alterações no metabolismo energético que interferem na composição das fibras musculares e no equilíbrio entre a síntese e degradação de proteínas (Panisset et al., 2016; Rabelo et al., 2017; Kern et al., 2017; Dumitru et al., 2017). A atrofia muscular esquelética é causada por uma série de fatores, incluindo doença, envelhecimento, lesão, decréscimos nutricionais e desuso. É caracterizada por uma diminuição na área da seção transversal do músculo, um declínio na capacidade geradora de força, diminuição das proteínas funcionais da massa muscular e perda da capacidade oxidativa, tornando o tecido menos resistente à fadiga (Theilen et al.; 2017). A atrofia por desnervação ocorre quando o nervo é interrompido e o tecido muscular não recebe mais sinais de estimulação do sistema nervoso. Este tipo de atrofia pode surgir de danos ao sistema nervoso central, como lesão medular, ou em lesões periféricas (Dumitru et al., 2017).

A sinalização do desequilíbrio muscular começa devido à falta de contração e estímulos musculares. Muitas vias contribuem para a atrofia muscular, mas, finalmente, o desequilíbrio da degradação excessiva de proteínas sem um aumento correspondente na síntese de proteínas resultará em uma perda líquida de tecido muscular. Atrofia também está correlacionada com disfunção mitocondrial. Muitas vias de degradação de proteínas e sinalização do apoptose celular são estimuladas pelo aumento dos níveis de espécies reativas de oxigênio, como ocorre na disfunção mitocondrial (Theilen et al.; 2017).

Quando a degradação de proteínas excede a síntese de proteínas ao longo do tempo, a atrofia ocorre juntamente com uma diminuição da função mitocondrial (Dumitru et al., 2017; Theilen et al.; 2017). As células sofrem uma morte celular programada (apoptose) devido a interações bioquímicas específicas que causam *blebbing* da membrana celular, condensação de cromatina e lise de fragmentos celulares, resultando na morte da célula. Quando o músculo esquelético não se contrai ao longo do tempo, como ocorre no desuso, observa-se um aumento no apoptose celular (Siu et al., 2009; Theilen et al.; 2017).

Na LM, a atrofia muscular ocorre predominantemente na fase aguda após LM (<3 meses após a lesão) (Gorgey and Dudley, 2007), em decorrência da ausência de recrutamento muscular voluntário. No primeiro mês após a lesão, a espessura muscular, medida por ultrassom, diminui em até 40%, sendo razoável indicar o período de até três meses como o "estágio inicial" da paraplegia por desuso (Taylor, 1993, Gorgey and Dudley, 2007). Apesar da condição de saúde adquirida, na maioria dos casos, o estado atrófico dos músculos pode ser parcialmente reversível se a ativação pela unidade motora tiver sido restaurada. Presumivelmente, o que determina estados atróficos é uma estreita relação entre estresse oxidativo e atrofia muscular por desuso (Oki et al., 2016; Rabelo et al., 2017; Kern et al., 2017).

A atrofia muscular e a capacidade reduzida de produzir torque contribuem para o desenvolvimento de incapacidade após a lesão. Pesquisadores encontraram que sujeitos com lesão medular incompleta produzem apenas 24% do torque extensor do joelho e 26% do torque flexor plantar em comparação a indivíduos sem lesão (Jayaraman et al., 2006). Estudos prévios demonstram reduções na área secção transversal média (CSA) medidos por biopsia em indivíduos após 6 semanas de LM, de 18% a 46% quando comparado a indivíduos saudáveis (Castro et al., 1999; Giangregorio et al., 2006). Estudo prospectivo de 24 semanas pós-LM desses pacientes, revelou mais reduções nos CSAs médios do gastrocnêmio e músculo sóleo de 24% e 12%, respectivamente (Castro et al., 1999; Giangregorio et al., 2006). Da mesma forma, 6 semanas a 24 semanas pós-lesão, as reduções médias nos CSAs do quadríceps, isquiotibiais e do músculo adutor foram de 16%, 14% e 16%, respectivamente. Reduções musculares podem resultar em diminuição da taxa metabólica e aumento do armazenamento de gordura intramuscular (Sedlock et al., 1990; Giangregorio et al., 2006). Da mesma forma, a recuperação da força dos membros inferiores é um dos determinantes da função de deambulação após a lesão medular. Sabe-se que 70% dos sujeitos com lesão medular incompleta é capaz de recuperar alguma forma de deambular - com ou sem órteses e dispositivos de auxílio

(Burns and Ditunno, 2001). Sendo a marcha é afetada pela espasticidade, coativação e pela fraqueza muscular (Krawetz e Nance; 1996; Rabelo et al., 2017).

A reabilitação requer o diagnóstico do nível e da gravidade da lesão por meio da identificação das zonas sensório-motoras íntegras, o que permite ao clínico traçar o tratamento e o prognóstico, bem como acompanhar a recuperação do sistema nervoso. A escala da ASIA é o método padronizado para essa avaliação (van Middendorp et al., 2011), mas a sua aplicação é limitada em pacientes não colaborativos e não fornece todos os dados necessários para o conhecimento das alterações fisiológicas e estruturais secundárias e para a escolha de determinadas abordagens terapêuticas. Diante disso, exames eletrofisiológicos e ultrassonográficos têm sido utilizados para suprir essa demanda (Curt & Dietz, 1999; Smith et al., 2017).

1.2. Avaliação da arquitetura muscular

O ultrassom tem sido reconhecido como uma importante ferramenta para o diagnóstico de diversas condições no tecido musculoesquelético (Zbojniewicz, 2014). A ultrassonografia é um método não invasivo amplamente utilizado na identificação de alterações da estrutura e morfologia musculotendínea (Grimm, 2013; Parry, 2015; Puthucheary, 2015) e que apresenta boa confiabilidade inter e intra-examinador (Tillquist, 2014; Baldwin, 2011; Dudley-Javoroski, 2010). Outros exames de imagem utilizados com esta finalidade são a ressonância magnética e a tomografia computadorizada, porém, a ultrassonografia tem a vantagem de não usar radiação, ser de baixo custo e ter exemplares portáteis (Walton et al., 1997; Dupont et al., 2001). A ultrassonografia permite a visualização do músculo, nervo e estruturas adjacentes e pode oferecer informações em tempo real no processo de perda de massa muscular devido à atrofia.

No músculo esquelético, as fibras se organizam basicamente em dois tipos, fusiforme e penado. No músculo fusiforme, como o Bíceps Braquial (BB), as fibras são longas e paralelas, conectando o tendão proximal ao distal. O músculo penado possui fibras curtas, como o Tibial Anterior (TA), com uma orientação oblíqua entre a aponeurose profunda até a superficial. Estas diferentes geometrias conferem diferentes funções primárias aos músculos, como velocidade de contração (fusiformes) e geração de força (penados), muito estudadas na biomecânica muscular. Para quantificar as diferentes geometrias musculares do corpo humano com o ultrassom, vários parâmetros chamados de arquitetura muscular são quantificados, como a espessura e ecogenicidade muscular,

do ângulo de penação e do comprimento fascicular (Parry, 2015; Puthucheary, 2015; Bunnell, 2015). Essas variáveis são úteis na avaliação da capacidade de realização de movimentos articulares, assim como um preditor da capacidade de geração de força (Ackland, 2012; Anderson, 2001).

A espessura do músculo está relacionada à área da seção transversa do músculo e é definida como o ponto com o maior diâmetro muscular (Blazevich, 2006; Franchi et al., 2018, Turton et al., 2019). A ecogenicidade muscular está associada à qualidade muscular, representando a composição da musculatura contra a infiltração de tecido não contrátil, como tecido adiposo ou fibroso, água e edema, sendo quantificado por análise em escala de cinza (Mayans et al., 2012; Strasser et al., 2013). Por sua vez, o ângulo de penação é definido como o ângulo entre a fibra muscular perpendicular ao eixo longitudinal das fibras musculares (Blazevich, 2006; Strasser et al., 2013). Se o músculo é penado, contém mais fibras em paralelo e, consequentemente, mais fibras contráteis. O comprimento fascicular, que é uma estimativa do comprimento da fibra muscular, é definido como o comprimento do fascículo e as aponeuroses superficiais e profundas e indica um intervalo de comprimento no qual o músculo é capaz de produzir ativamente força (Lieber and Fridén, 2000; Blazevich, 2006).

Em uma imagem de ultrassom, o músculo esquelético pode ser facilmente distinguido de outras estruturas como a gordura subcutânea e o osso (Pillen et al. 2006). O músculo esquelético saudável possui pouco tecido fibroso e isto o caracteriza como uma estrutura hipoecóica (mais preto) (Peetrons, 2002). Para o mesmo músculo, a orientação longitudinal e transversal do transdutor resulta em diferentes imagens de ultrassom, onde diferentes parâmetros de arquitetura podem ser melhores definidos. As imagens obtidas através da orientação longitudinal do transdutor permitem a análise de algumas propriedades do músculo como o ângulo de penação e o comprimento fascicular. Entretanto, a recomendação apropriada para a orientação da sonda que deve ser usada para uma melhor análise das imagens em músculos esqueléticos ainda não está estabelecida (Blazevich, 2006).

Com as medidas de espessura e ecogenicidade é possível prever alterações na força muscular e na funcionalidade (Perry et al., 2015). Existe uma relação comprovada entre força e espessura do músculo quadríceps femoral em humanos (Chi-Fishman et al., 2004; Sarwal et al., 2015). Dessa forma, o exame pode fornecer subsídios para identificar potenciais respondedores à reabilitação ainda na fase precoce da injuria.

1.3. Avaliação da arquitetura tendínea

Os tendões e os ligamentos são estruturas especializadas, com elaborada arquitetura anatômica, que desempenham papel fundamental na locomoção. Os tendões transferem a força gerada pela contração muscular para os ossos, através das articulações, promovendo o movimento (Ingraham et al., 2003). Os tendões possuem a capacidade de se adaptar às diferentes cargas mecânicas que lhes são aplicadas e por consequência alteram sua composição, propriedades mecânicas e estruturais (Wang et al., 2006). Mesmo o desuso a curto prazo pode causar alterações nas características teciduais do tendão, como na sua viscoelasticidade, levando a uma menor rigidez desta estrutura (Hannafin et al., 1995; Kubo et al., 2004; Matsumoto et al., 2003). Entretanto, a literatura ainda carece de estudos que correlacionem o desuso a curto prazo com a redução da espessura do tendão, especificamente o tendão do quadríceps.

A maior parte do conhecimento sobre os efeitos do desuso na mecânica dos tendões provém de estudos com animais e durações relativamente curtas de descarga experimental, com duração de 3 a 12 semanas. Entretanto, alguns fatores tornam difícil a aplicabilidade dos achados de trabalho animal in vitro para tendões humanos in vivo, como: diferenças no nível de resistência mecânica do tendão, diferenças na carga fisiológica entre os tendões de um determinado animal e diferenças na atividade desempenhada pelos animais. Os avanços na ultrassonografia, no entanto, permitiram avaliar in vivo os efeitos do desuso nas propriedades mecânicas do tendão humano intacto (Carroll et al., 2008; Magnusson et al., 2008; Hsin-Yi Liu, 2013).

Os tendões são considerados fundamentais na transmissão de força contrátil para manutenção da postura e/ou produção do movimento. Estruturalmente, o tendão é revestido por uma quantidade de tecido conjuntivo frouxo, denominado paratendão, que permite a livre circulação do tecido. A segunda camada, denominada epitendão, é contínua com o paratendão em sua superfície externa e, com o endotendão, na sua superfície interna, agrupando os endotendões de forma organizacional. Finalmente, o endotendão envolve cada fibra individualmente e conduz vasos sanguíneos e nervos (Sharma and Maffulli, 2005; O'Brien, 2005). Em relação à sua constituição, os tendões apresentam uma grande matriz extracelular, extremamente resistente a forças de tração e compressão e uma quantidade relativamente pequena de células, responsáveis pela manutenção dessa matriz (Birch, 2007; Lavagnino et al., 2015). A matriz extracelular tendínea é composta por aproximadamente 70% de água, com boa parte associada aos

glicosaminoglicanos, proteínas importantes para a viscoelasticidade tendínea, garantindo redução de energia metabólica durante a locomoção (Kjaer, 2004), além de proteoglicanos, glicoproteínas de adesão e proteínas fibrosas (colágeno e elastina) (Kjaer, 2004; Wang, 2006). O principal constituinte dessa matriz é o colágeno, que representa 60-85% do peso seco do tendão, arranjado paralelamente na direção de aplicação da força do músculo, em fibras resistentes às tensões, garantindo a integridade estrutural do tecido (Kjaer, 2004; Wang, 2006).

Estudos in vitro (Heinemeier e Kjaer, 2011) foram as primeiras evidências de que, assim como nos músculos, as dimensões e propriedades mecânicas dos tendões se alteram dependendo do nível de atividade a que são submetidos, incluindo o desuso, que acarreta redução da rigidez tecidual, da área de secção transversa e espessura do tendão (Maganaris et al., 2006; Ninomyia et al., 2007; Couppe et al., 2008; Hogaboom et al., 2016). Kubo et al. (2004) relataram alterações significativas, com redução de até 30% na rigidez e na histerese das estruturas do tendão humano nos extensores do joelho após 20 dias de desuso. Períodos mais longos de desuso (maiores que 90 dias) produziram redução mais significativa na rigidez do tendão, atingindo o nível de 60% (Kubo et al., 2004; Reeveset al., 2005). Entretanto, apenas um único estudo documentou alterações nos tendões após a LM, sugerindo que a área transversal do tendão pode diminuir após LM e que longos períodos de desuso (mais de 90 dias) produziram maiores reduções na rigidez do tendão (Maganaris et al. 2006). Isso sugere que o desuso severo pode comprometer seriamente a integridade do tendão, predispondo-o a rupturas e micro lesões. São escassos estudos que abordem o comportamento tendíneo em pacientes com LM através da ultrassonografia (Dudley-Javoroski, 2010).

1.4. Teste de Eletrodiagnóstico por Estímulo na Lesão Medular

Os exames eletrofisiológicos mais utilizados na busca por comprometimentos neuromusculares são os estudos da condução nervosa, a eletromiografia e os potenciais evocados, os quais fornecem informações detalhadas sobre o sistema sensório-motor, desde as regiões corticais às unidades motoras ou terminações sensoriais. A maioria destes testes não é invasiva e fornece evidências sobre como a condução nervosa em segmentos afetados da medula espinhal melhora espontaneamente ou por meio de intervenções terapêuticas, bem com mostra alterações secundárias (Petersen et al., 2017). Sob esse prisma, foi mostrado que o desfecho clínico e o retorno à marcha após uma LM

podem ser preditos pela avaliação do potencial evocado motor do músculo tibial anterior (Petersen et al., 2012), enquanto que Van De Meent et al. (2010) e Riley et al. (2011) mostraram por meio do potencial de ação motor composto que a LM gera déficits consideráveis nos nervos periféricos de motoneurônios localizados abaixo dos seguimentos da lesão.

Entre os vários meios de avaliação das alterações eletrofisiológicas, está o eletrodiagnóstico de estímulo (SET). Além do seu uso para fins de avaliação, o SET é o único recurso disponível para estabelecer as condições ideais de eletroestimulação terapêutica, garantindo o uso do pulso elétrico mais adequado para o tratamento de uma lesão específica (Ervilha et al., 1997). É um método não invasivo de monitoramento de condições neurais, que se fundamenta na verificação do padrão de contração muscular produzida por vários parâmetros de Estimulação Elétrica Neuromuscular (NMES) (Ervilha et al., 1997), que mensura a reobase, cronaxia, acomodação e índice de acomodação (Paternostro-Sluga et al., 2002; Schuhfried et al., 2005; Lee et al., 2013). A reobase é a menor intensidade de corrente em miliampéres (mA) necessária para produzir uma contração mínima do músculo, com pulso retangular de duração infinita e intervalo entre os pulsos igual a 2000 ms. A cronaxia é a menor duração de pulso, necessária para produzir uma contração mínima do músculo com um pulso quadrado, intensidade igual ao dobro da reobase com intervalo entre os pulsos de 2000 ms (Irnich, 2010). A acomodação é a menor intensidade necessária para produzir uma contração muscular, sendo o pulso de formato exponencial e largura infinita, ou seja, é a propriedade que o músculo responde apenas com altas intensidades a pulsos de crescimento lento (exponencial). Já o índice de acomodação é a relação entre a acomodação e a reobase (Paternostro-Sluga, 2002).

A contração do músculo normalmente inervado é forte e ocorre com um estímulo de baixa duração, enquanto que o músculo desnervado requer maior duração de corrente e apresenta contração lenta e involuntárias. Os resultados desse teste permitem elucidar o diagnóstico diferencial entre lesão do sistema nervoso periférico e lesão do sistema nervoso central (Holland, 2012). Seguindo este princípio, a desnervação muscular resulta na dificuldade de despolarização das fibras dos músculos e, consequentemente, gera um aumento da reobase e da cronaxia, pois o limiar do sarcolema é maior que o do nervo periférico.

A cronaxia é o parâmetro de SET sensível e útil a ser usado para avaliar o processo de recuperação/regeneração nervosa, permitindo a escolha da duração do pulso para

estimulação (Paternostro-Sluga et al., 2002). A corrente induzida em tecidos biológicos deve ter amplitude e duração suficientes para levar células excitáveis a uma tensão suficiente para evocar um potencial de ação (Schuhfried et al., 2005). Com base na mensuração da cronaxia, o ajuste dos parâmetros na NMES para a estimulação do músculo pode ser otimizado, onde a identificação do valor da cronaxia indica a duração do pulso ideal para estimular o tecido muscular (Geddes et al., 2004; Schuhfried et al., 2005). Um aumento da cronaxia é indicativa de desnervação da fibra muscular (Fernandes et al., 2016). Os distúrbios eletrofisiológicos neuromusculares (NED) também podem ser diagnosticados pelo SET, que apresenta sensibilidade variando de 88% a 100% quando comparado à eletromiografia por agulha. Valores de cronaxia ≥1000 μs caracterizam a presença de NED (Paternostro-Sluga et al., 2002).

Estudos anteriores de condução nervosa e eletromiografia invasiva (Rosen et al., 1969; Campbell et al., 1991; Kirshblum et al., 2001; Shin-Yi Lin et al., 2007) encontraram atividade espontânea nos testes eletromiográficos em pacientes com LM. Eles descobriram, evidenciado por fibrilações e potenciais de ondas agudas positivas no exame, tanto nas fases aguda e crônica da lesão, indicando fibras musculares desnervadas e consequentemente alterações de excitabilidade muscular em indivíduos com lesões medulares completas e incompletas. Kirshblum et al. (2001) também demonstrrarm uma redução na velocidade de condução nervosa e no potencial de ação muscular quando comparados a um grupo pareado nos músculos vasto medial, iliopsoas, tibial anterior, gastrocnêmio medial e paraespinhal lombar. A atividade espontânea foi registrada em pelo menos um dos músculos testados em 92% dos indivíduos. O maior efeito na amplitude da forma de onda nervosa sugere um envolvimento axonal predominante. (Kirshblum et al., 2001; Shin-Yi Lin et al., 2007).

As alterações da excitabilidade podem não apenas prejudicar a condução nervosa, como os protocolos clínicos do NMES. Arpin et al. (2019) sugerem que, indivíduos com LM, que não respondem aos protocolos convencionais de NMES, se beneficiariam de larguras de pulso maiores (500 e 1.000 μs), causando maior ativação central no recrutamento de mais unidades motoras sem alterar a velocidade média de condução das fibras musculares. Pacientes com NED podem precisar de NMES com largura de pulso >1000 μs. Alguns autores sugerem que a largura de pulso usada para tratar pacientes com NED deve ser definida com base em cronaxia (Silva et al., 2017; Arpin et al., 2019). O tratamento NMES com base na cronaxia pode provocar contrações mais vigorosas e, assim, pode melhorar a eficácia do tratamento (Silva et al., 2017).

Da mesma forma, Guimarães et al. (2016) também sugeriu que a não responsividade à NMES pode não apenas estar associada à atrofia muscular e disfunções do nervo motor devido ao tempo de lesão, mas também a danos nas fibras motoras descendentes de neurônios motores localizados no córtex motor (síndrome de desconexão). Indivíduos não responsivos à NMES podem apresentar danos às vias motoras periféricas, em outras palavras, localizadas nas raízes nervosas e/ou nos axônios dos neurônios motores (Guimarães et al., 2016). Isso precisa ser levado em consideração no projeto de protocolos de estimulação elétrica para fins de reabilitação e experimental

2. OBJETIVOS

2.1. Objetivo Geral

Detectar alterações na função neuromuscular e na estrutura musculoesquelética nos músculos paralisados com inervações preservadas em indivíduos com lesão medular traumática e comparar com pessoas sem lesão.

2.2. Objetivos Específicos

3.1.1. Analisar a excitabilidade neuromuscular, considerando a cronaxia, reobase, acomodação e índice de acomodação, nos músculos bíceps braquial, quadríceps e tibial anterior de indivíduos com lesão medular traumática;

3.1.2. Analisar a arquitetura muscular, incluindo espessura, ecogenicidade, ângulo de penação e comprimento fascicular nos músculos, bíceps braquial, quadríceps e tibial anterior em indivíduos com lesão medular traumática;

3.1.3. Analisar a arquitetura tendínea, compreendendo a espessura e a ecogenicidade, nos tendões quadricipital e patelar em indivíduos com lesão medular traumática;

3.1.4. Avaliar a reprodutibilidade inter-observador do SET e ultrassonografia musculotendínea em indivíduos com lesão medular traumática.

2.3 Hipótese

A presença de atrofia muscular crônica em indivíduos com LM promove alterações na arquitetura muscular, observadas pela redução da espessura, aumento da ecogenicidade e diminuição do ângulo de penação e comprimento fascicular, associado a alterações na excitabilidade neuromuscular relacionada à presença de NED.

3. MANUSCRITO

NEW PERSPECTIVES IN A CROSS-SECTIONAL STUDY ON MUSCLES AND TENDONS IMPAIRMENTS DUE TO NEUROMUSCULAR MALADAPTED FUNCTION FOUND IN CHRONIC SPINAL CORD INJURY

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1. Introduction

Central nervous system diseases include brain or spinal cord injury (SCI) impairments and may result in movement disorders almost always manifested by paralyzed muscles with preserved innervations (1). Muscle atrophy occurs predominantly in the acute phase after SCI (<3 months post-injury) (Gorgey and Dudley, 2007). In the first month after the injury, muscle thickness, measured by ultrasound, decreases by up to 40%, it being reasonable to indicate the period of up to three months as the "early stage" of disuse paraplegia (2, 3). Ultrasound allows the visualization of the muscle, nerve, and adjacent structures and can offer real-time information in the process of muscle wasting due to atrophy after SCI (3, 4).

Muscle architecture evaluated by ultrasound provides information on the thickness, echogenicity, pennation angle, and fascicular length (5-7). Muscle thickness is related to the muscle cross-sectional area and is defined as the point with the largest muscle diameter (8-11). Muscle echogenicity is associated with muscle quality, representing the composition of the musculature against the infiltration of non-contractile tissue, such as adipose or fibrous tissue, water, and edema, being quantified by gray-scale analysis (12, 13). In turn, pennation angle is defined as the angle between the muscle fiber perpendicular to the longitudinal axis of the muscle fibers (8, 13). If the muscle is pennate, it contains more fibers in parallel and, consequently, more contractile fibers. The fascicular length, which is an estimate of the muscle fiber length, is defined as the fascicle length and the superficial and deep aponeuroses and indicates a length interval in which the muscle is capable of actively producing force (8, 14).

Likewise, tendons can also be affected after SCI for being a very relevant structure that allows muscle-bone load transmission. With advances in ultrasound, the effects of disuse on the mechanical properties of the human tendon have been studied *in vivo* (15-17). However, only a single study had documented changes in tendons after SCI, suggesting that the tendon CSA may decrease after SCI as well as after long disuse periods (over 90 days) produced greater reductions in tendon stiffness (4). These facts indicate that SCI causes substantial deterioration of the structural and material properties in the tendon, predisposing it to ruptures and microlesions (4, 18).

Previous studies of nerve conduction and invasive electromyography found that denervated muscle fibers and consequently alterations of muscle excitability in individuals with complete and incomplete SCI (19-22). Kirshblum et al. (21) also demonstrated a reduction in nerve conduction velocity and muscle action potential when

compared to a healthy group in the vastus medialis, iliopsoas, anterior tibialis, medial gastrocnemius, and lumbar paraspinal muscles. Spontaneous activity was recorded in at least one of the muscles tested in 92% of subjects. The greatest effect on nerve waveform amplitude suggests a predominant axonal involvement. However, there is no definitive finding on neuromuscular function changes observed in individuals with chronic SCI (21, 22).

Among many modalities of evaluating peripheral nerve lesions, the Stimulus Electrodiagnosis Test (SET) is a non-invasive examination that quantifies the responses evoked by the nerve and muscle using a neuromuscular electrical stimulation (NMES) that measured the rheobase, chronaxie, accommodation, and accommodation index (23-25). Needle electromyography has been indicated as a relevant test to determine peripheral nerve injury level and their severity (26). Nonetheless, the feasibility of this test can be low due to the considerable cost, need for a skilled physician, and the inherent risk of an invasive test (27). Neuromuscular Electrophysiological Disorders (NED) can also be diagnosed by SET, which presents sensitivity ranging from 88% to 100% when compared to needle electromyography (23). Chronaxie needs to be taken into consideration in the design of electrical stimulation protocols for rehabilitation and experimental purposes (23, 27, 28). Besides, the patterns of muscle atrophy and other mechanisms for non-responsivity to neuromuscular function parameters have not yet been elucidated after SCI.

Therefore, the purpose of this study was to detect changes in musculoskeletal structure and neuromuscular function of the paralyzed muscles of individuals with SCI and compare to able-bodied people. We hypothesized that individuals with SCI would have a reduction in muscle thickness, an increase in echogenicity, and decreases in both pennation angle and fascicular length than able body participants accompanied by the presence of NED. Also, SCI would present a reduction in tendon thickness and an increase in echogenicity.

2. Methods

2.1. Participants

An observational cross-sectional case-control study was designed to investigate muscle and tendon structure architecture and NED in individuals with chronic SCI. Center for Training and Special Physical Education (CETEFE) in Brasília/DF hosted participants and researchers during all steps of the data collection. The participants were informed of the procedures, purposes, benefits, and risks of the study and signed an informed consent form. The research was approved by the Human Research Ethics Committee at the University of Brasilia/Faculty of Ceilândia (protocol number: 82655418.2.0000.8093) and was conducted following the Helsinki Declaration (1975).

A convenience sample of 30 participants of both sexes, between 18 and 60 years of age, volunteered to participate. Complete or incomplete SCI participants with injury time over 3 years were included, according to a previous clinical diagnosis and the American Spinal Injury Association (ASIA) (29), and injury level below C5, where there is no impairment of brachial biceps innervation. We recruited an able-bodied group with similar anthropometric and demographic outcomes, excluding participants with a body mass index (BMI) $\geq$ 35 kg/m^2; pregnancy; pain, lesion, deformity or amputation in the regions to be examined; conditions affecting muscle morphology or neuromuscular excitability; ankylosing spondylitis, rheumatoid arthritis, type 2 diabetes mellitus, hypercholesterolemia, associated neuromuscular disease, severe heart disease (Gea, 2013), isolated obstructive pulmonary disease; cognitive impairment, chemical dependency, psychiatric illness, or behavioral problems that would make cooperation with procedures difficult (18).

2.2. Study flow

Subjects included in the study underwent stimulus electrodiagnostic testing to record the chronaxie, rheobase, accommodation, and accommodation index; as well as to take values of muscle ultrasound parameters (thickness, pennation angle, fascicle length, and echo intensity) from the biceps brachii (BB), rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), and anterior tibialis (TA). Regarding tendon architecture parameters (thickness and echogenicity), measures from the quadriceps and patellar tendons were taken. We also performed tests on the BB muscle to confirm whether individuals with SCI have intact upper limbs. We preceded all data collection bilaterally for healthy and impaired muscles. During the assessment, the subjects were comfortably

positioned, supine, on a therapeutic stretcher, with a pillow under their head and a semi-rigid roll under the popliteal fossa to maintain their knees and hips at 30º flexion. The neutrality of hip rotation was achieved with a cotton band around the knees. The upper limbs were in external rotation and 45º abduction.

2.3.Stimulus electrodiagnostic testing

A universal pulse generator (Dualpex 071, Quark Medical LTDA, Piracicaba, Brazil) was used to assess the five target muscles by mean of a reference electrode (Anode) with an area of 100 cm^2, placed on the ankle for all measurements. With an active pen electrode (Cathode) with an area of 1 cm^2, we located the motor-points as previously described (30). The minimal current intensity necessary to reach the neuromuscular excitability threshold applied with a rectangular pulse with an infinite duration (e.g., 1 s), the rheobase, varied from 0 up to 69 mA (the maximum intensity allowed by the stimulator), with increments of 1 mA until the point at a slight but visible muscle contraction appeared. A rectangular pulse width of 1 second and a rest interval of 2 seconds defined the protocol of stimulation during the assessments (23). In turn, the shortest pulse duration required to reach the neuromuscular excitability threshold by a current with twice the intensity of the rheobase represents the chronaxie and values higher than 1000 µs indicated NED as proposed by Paternostro-Sluga et al. (2002). The stimulator allowed us to apply pulse width increasing from 20 µs to 1s, using 100 µs increments until 1000 µs, followed by 1000 µs increments from this point (23). We applied an exponential pulse monopolar current with a pulse width of 1000 µs to define the accommodation values, increasing the electrical current from 0 to 69 mA with 1mA increments until the visible muscle contraction. The accommodation values allowed us to calculate the accommodation index (AI) by the ratio between accommodation and rheobase (AI = accommodation/rheobase) (23, 28).

2.4. Muscle and tendon ultrasound

Muscle thickness and echogenicity were measured using a portable B-mode ultrasound device, M-Turbo® (Sonosite, Bothwell, WA, USA), with a frequency linear array probe (HFL38, bandwidth: 13-6MHz, maximal scan depth: 6 cm). Participants were placed in the supine position with their knees in hip flexion of 35° and neutral rotation, 10 minutes before the onset of recording. A water-soluble transmission gel was applied to the measurement site, and a 7.5 MHz ultrasound probe was placed perpendicular to the

longitudinal plane of the muscle, keeping it parallel to the direction of the muscle fascicles, while not depressing the skin. Muscle thickness was carried out in the five muscles (BB, RF, VL, VM, and TA). Probe placements and measurements were performed according to previous recommendations (8, 31, 32).

The BB muscle (including the underlying biceps brachii muscle) was measured between the uppermost part of the bone echo of the humerus and the superficial fascia of the biceps; the probe was placed at two-thirds of the distance from the acromion to the cubital fossa. The RF (which includes the rectus femoris and vastus intermedius muscles), VL, and VM were evaluated, respectively, at the percentages 50%, 60%, and 80% of the distance between the anterior superior iliac spine and the superior border of the patella, starting from proximal to distal, adapted from Blazevich et al. (8). The RF was visualized in the anterior aspect of the thigh, the VL was visualized by moving the transducer laterally 5 cm from the midline, and the VM was visualized with the transducer 3 cm in the medial direction of the thigh. The thickness of the RF, VL, and VM muscles was considered as the distance between the superficial fascia and the deep fascia of the respective muscles (8). The TA was assessed between the interosseous membrane (next to the tibia) and the superficial fascia of the tibialis anterior; the probe was placed at one-quarter of the distance from the inferior aspect of the patella to the lateral malleolus (Figure 1).

Ultrasonographic evaluation of the quadriceps (TQ) and patellar (TP) tendons was performed. The probe was placed 3 cm proximal to the superior pole of the patella for the quadriceps tendon (33, 34) and at 25%, 50%, and 75% of the length of the patellar tendon. The patellar tendon length was measured between the deep insertion in the patella and the deep insertion in the tibial tuberosity (35). These landmarks were easily visible on the ultrasonographic image as hyperechogenic regions in bone insertion. The raters positioned the probe perpendicularly and transversally (axial plane), maintaining constant depth, gain, and ultrasound settings throughout the data collection period. The thickness measurement was performed considering the maximum distance between the tendon contours, including the peritendon (33, 34, 36).

Three images were obtained for each participant. In order to perform the analysis of muscle and tendon thickness, pennation angle, and fascicle length the software Image J (Image J version 1.43, National Institutes of Health, USA) was used. Pennation angle was considered as the angle formed between the deep aponeurosis and the fascicle that rises from the same aponeurosis. For fascicle length, corrections were made according to the

recommendations of Blazevich et al. (8). For muscle and tendon echogenicity, a region of interest was selected in each muscle using the tracing technique to include all visible muscle in the ultrasonographic image without any bone or surrounding fascia (37). The mean echogenicity of the region of interest was calculated (8-bit resolution, resulting in a number between 0 and 255, where black = 0, white = 255) and averaged over the three measurements per muscle (37, 38).

2.5. Reproducibility of electrodiagnosis and ultrasound

Two examiners were involved in the study, both physiotherapists, with two years of experience in sonographic evaluation and who had trained extensively for six months in obtaining images using the study method, as well as performing electrodiagnosis. SET and ultrasound data from each participant were obtained at the same time of day by the evaluators, who did not have access to each other's measurements. To verify the reproducibility of the SET, each examiner individually searched for the motor point and other measurements.

3. Statistical analysis

The quantitative variables were respectively expressed as mean and standard deviation (mean ± SD) or frequency distribution. The Shapiro-Wilk test was used to investigate whether the data were normally distributed. As all included variables were normally distributed, parametric statistics were performed, except the chronaxis, where median and interquartile range were used. Clinical data were collected individually before ultrasound and SET collections. The severity and level of the injury was categorized according to the ASIA. The muscle architecture (thickness, pennation angle, fascicle length, and echo intensity) were assessed by two-way ANOVA with “Type of muscle” (five levels: BB, RF, VL, VM, and TA) and “groups” (two levels: control and spinal cord injury groups) as factors. Chronaxie was assessed by Kruskal Wallis test. For all tests, the significance level was set at $p<0.05$. *Post-hoc* analyses (Tukey HSD tests) were performed when appropriate. Tendon architecture (thickness and echo intensity) was assessed using the unpaired t-test. To assess the presence or absence of NED, we used the Mann-Whitney test, represented by bar and boxplot graphs. Additionally, statistical power and effect sizes were calculated. Sperman correlation was used to assess correlations between the chronaxie and US variables in muscles and tendons. For the intraclass correlation, the sample consisted of 11 subjects, where three images of each

variable were obtained and calculated through the mean of the measurements of the two evaluators. To interpret the magnitude of the correlation coefficients (ICC) was classified by the scale suggested by Lee et al (2012): 0 (absence), 0–0.19 (poor), 0.20–0.39 (weak), 0.40–0.59 (moderate), 0.60–0.79 (substantial), and > 0.80 (almost complete). Effect sizes were determined using partial eta squared (η_ρ^2) for ANOVA. Effect sizes and statistical power were calculated. Effect size was determined using partial eta squared (ηρ2): small (ηρ2 = 0.01), medium (ηρ2 = 0.06), and large (ηρ2 = 0.14) effects. All statistical analyses were performed using the software STATISTICA StatSoft Inc., Tulsa, Oklahoma, USA). The significance threshold was set at $P<0.05$ for all procedures.

4. Results

Fifteen individuals with SCI participated in this study, 80% male, with a mean age 36.8 ±8 years, weight 69.7 ±11.8 kg, height 1.72 ±0.08 cm. In addition, fifteen able-bodied individuals participated, 80% male, mean age 32.1 ± 6.5 years, weight 77.5 ±13.0 kg, height 1.70 ±0.09 cm. There were no significant differences for groups' demographic data. The characteristics of the SCI group are shown in Table 1. No selected individuals met the exclusion criteria established in this study.

Table 1: Characterization of the clinical data of the SCI (n =15).

Time since injury (years)	14.6 ± 5.5
Etiology	
Gunshot wound	26.7%
Auto accident	33.4%
Falls	13.3%
Diving	26.7%
Injury Level	
Cervical	26.7%
Thoracic	53.5%
Lumbosacral	19.8%
Sport practiced	
Badminton	21,4%
Powerlifting	28,6%
Wheelchair rugby	28,6%
Tennis	7,1%
Archery	7,1%
Sailing	7,1%
ASIA impairment scale (n =11)	
A	70%
B	10%
D	20%

Data expressed as mean ± SD and proportion. ASIA, American Spinal Injury Association.

4.1.Muscle architecture

There were no significant differences in muscle thickness ($p<0.796$), echogenicity ($p<0.946$), pennation angle ($p<0.888$), or fascicle length ($p<0.261$) when comparing ultrasound variables between the right and left sides in SCI. Regarding muscle thickness, the comparison between the groups showed a significant muscle *versus* impairment interaction ($F=2.96$, $p<0.022$, power=0.77, $\eta\rho2=0.095$, ICC 0.91 ($p<0.05$), Figure 2), there was no difference in the BB muscle between the control and SCI groups ($p>0.068$). In the RF ($p<0.00015$), VL ($p<0.00016$), VM ($p<0.00016$), and TA muscles ($p<0.0007$), significant differences were observed when comparing the thickness between the able-bodied subjects and SCI. The comparison of the muscle thickness of the BB (muscles with preserved innervation in the sample) showed significant results in the muscles: RF ($p<0.00018$), VL ($p<0.00019$), VM ($p<0.00015$), and TA ($p<0.00016$).

A similar significant muscle *versus* impairment interaction was observed for muscle echo intensity between groups ($F=6.74$, $p<0.0001$, power=0.99, $\eta\rho2=0.19$, ICC 0.90 ($p<0.05$), Figure 2). There was no difference in the BB muscle between the groups ($p>0.999$). In the RF ($p<0.00072$), VL ($p<0.00015$), VM ($p<0.00038$), and TA ($p<0.00017$) muscles, there was a significant difference when comparing the echo intensity between the able-bodied subjects and SCI. The comparison of the biceps brachii of the SCI, as well as the muscular thickness, presented significant results in the muscles: RF ($p<0.016$), VL ($p<0.00015$), VM ($p<0.012$), and TA ($p<0,00015$).

In the pennation angle, the comparison between groups showed a significant interaction between muscle versus impairment ($F=5.52$, $p<0.0001$, power=0.93, $\eta\rho2=0.16$, ICC 0.89 ($p<0.05$), Figure 2). There was a significant difference when comparing the pennation angle between the able-bodied subjects and SCI in the muscles: RF ($p<0.0014$), VL ($p<0.0001$), VM ($p<0.0001$), and TA ($p<0.032$). For the fascicle length, the comparison between the groups showed a statistically significant muscle *versus* impairment ($F=2.71$, $p<0.0497$, power=0.99, $\eta\rho2=0.24$, Figure 2). A significant difference was observed when comparing fascicular length between the able-bodied and SCI in the muscles: RF ($p<0.0159$) and TA ($p<0.0450$). Significant differences were found in the comparison of the RF muscle with the VM ($p<0.0001$), VL ($p<0.0167$), and TA ($p<0.0001$), as shown in Figure 2.

4.2.Tendon architecture

There were no significant differences in tendon thickness ($p<0.927$) and echogenicity ($p<0.403$) between the right and left sides of the SCI. In the tendon architecture, there was a significant difference in the TQ thickness variable when compared to the able-bodied ($p<0.046$, power=0.72, effect size D=0.84, Figure 4). There were no significant differences in the comparisons between groups of variables: TQ echogenicity ($p<0.090$, power=0.57, effect size D=0.68), TP echogenicity ($p<0.067$, power=0.60, effect size D=0.71), and TP thickness ($p<0.101$, power=0.51, effect size D=0.63), as shown in Figure 4. For the tendon ultrasound variables, the following ICCs were found: 0.89thickness and 0.93 echogenicity for quadriceps tendon ($p<0.05$), 0.92 for thickness and 0.96 echogenicity for patellar tendon ($p<0.05$).

4.3. Neuromuscular electrophysiological disorders

Regarding chronaxie values, significant differences were demonstrated between muscle versus impairment ($F=15.58$, $p<0.0003$, power=0.97, $\eta\rho2=0.16$, ICC 0.87 ($p<0.05$), Figure 4). In the able-bodied group, there was no difference between the chronaxie when comparing the different muscles. Between the SCI and able-bodied groups, significant differences were found in the RF ($p<0.000$), VL ($p<0.000$), and VM ($p <0.009$) muscles. Comparison of the BB chronaxie of the SCI showed significant results in the muscles: RF ($p<0.00015$), VL ($p<0.00016$), VM ($p<0.00020$), and TA ($p<0.0032$). Regarding the accommodation index, no significant results were found between the groups in the different muscles ($p>0.184$).

The prevalence of NED between the able-bodied and SCI groups is shown in Figure 5. The SCI had an prevalence of NED of 33.3% (5/15) in the RF and VL muscles and 26.6% (4/15) in the VM and TA muscles, Figure 4. The BB did not present NED in the SCI or the control.

Significant linear correlations were found between the chronaxie and ultrasound variables, as shown in Figure 4; negative correlations between chronaxie and muscle thickness in RF (P = - 0.590, p = 0.001), VL (P = - 0.598, p = 0.000), and VM (P = - 0.572, p = 0.001), in Figure 5A. Positive correlation between chronaxie and muscle echogenicity in RF (P = 0.612, p = 0.000), VL (P = 0.663, p = 0.000), VM (P = -0.437, p = 0.016) and TA (P = 0,409, p = 0.025), in Figure 5B. Negative correlations between chronaxie and penetration angle in RF (P = -0.549, p = 0.002), VL (P = -0.495, p = 0.005), and VM (P = - 0.609, p = 0.000), in Figure 5C.

Significant positive linear correlations were found between quadriceps tendon thickness and muscle thickness in VL (P = 0.441, p = 0.015), VM (P = 0.659, p = 0.000), and TA (P = 0.698, p = 0.000). Positive correlations were also found between patellar tendon thickness and muscle thickness in VL (P = 0.420, p = 0.021), VM (P = 0.599, p = 0.000), and TA (P = 0.650, p = 0.000).

5. Discussion

The main findings of this study are in accordance with the initial hypothesis that SCI promotes alterations in the muscle and tendon architecture, observed through the reduction in thickness, increase in echogenicity, and decrease in both pennation angle and fascicular length. Monitoring changes in muscle and tendon architecture associated with NED alterations may lead to early detection and better quantification of muscle loss, as well as appropriately targeting appropriate rehabilitation of SCI patients.

It was demonstrated that SCI participants presented lower values of thickness, pennation angle, and fascicle length, and higher values of echogenicity when compared to able-bodied participants. The current study found significantly lower values in RF, VL, VM, and TA muscle thickness (reduction of nearly 40% in comparison with healthy muscles). Similar results were observed in studies which used magnetic resonance imaging techniques in individuals with SCI (39-41). In addition, there is growing interest in mensuration of muscle quality alterations (echogenicity), particularly in the process of muscle disuse. An increase in echogenicity was demonstrated in this current work, resulting in whiter or sparkler muscle (hyperechoic) (42, 43). It is possible that the increased echogenicity could be related to the type of musculotendinous involvement which SCI individuals are exposed to, such as prolonged immobility or atrophy caused by disuse, systemic inflammatory response, fat deposition on musculature, and muscle tissue replacement by fibrosis (6, 44).

In the current study, the loss of muscle thickness was correlated with a significant reduction in the pennation angle of fascicular musculature and fascicle length. Regarding the pennation angle, our results showed lower values in the RF, VL, VM, and TA muscles, when compared to the paired group. There was no evidence that SCI subjects had muscle fascicles or pennation angle shorter. Some studies, such as the Morse et al. revealed a decay of 12% in the gastrocnemius muscle pennation angle in older individuals (45). Turton et al. demonstrated a decrease in VL pennation angle, after 5-10 days of prolonged immobility in intensive unit therapy and with the use of invasive mechanical ventilation (11). Diong et al. (46) demonstrated that pennation at fascicle length in gastrocnemius muscle was significantly less in SCI than control subjects, corroborating with the present study. These findings are consistent with other data showing that atrophy causes a reduction in pennation (11,45) and that the contribution of pennation to effective fascicle length is small. Interestingly, the pennation angle is related to the ability to store more contractile components at a given volume and can generate greater force with angles up to 45 degrees (47). If the reduction in the pennation angle leads to decay in force generation, this may help to explain muscle weakness associated with functional modifications due to the injury level in individuals with SCI (47). In addition, we demonstrated that in RF, VL, VM, and TA muscles, the muscle thickness and pennation angle are related to and support previous studies which stated that muscles with greater thickness could generate more force due to their greater pennation angles and, consequently, larger cross-sectional areas (11, 47). Since pennation angle is directly related to the force generation properties (14, 48), our results suggest that the pennation angle should be monitored in individuals with SCI.

Although muscle adaptations after SCI have been extensively studied (39, 49-51), tendon remodeling remains largely unknown in this population. Dudley-Javoroski et al. (18) evaluated the patellar tendon and Achilles tendon in individuals with SCI but did not detect differences in tendon thickness. Individuals with chronic SCI were reported to have a 17% lower patellar tendon CSA as well as tendon stiffness and Young's modulus diminished by 77% compared to the able-bodied subjects (4). We did not assess the deterioration in tendon biomechanics (tendon stiffness and Young's modulus) due to difficulty in performing force measurements in our participants, which limit the comparison between our results to the Maganaris et al. (4). It is possible to suggest that as these tendons become stretched with prolonged use of a wheelchair, which keeps the knee flexed continuously at 90 degrees and this particular position can benefit the patellar

tendons of people with SCI (4). Future research is needed to elucidate the role of the joint position in the deterioration of human tendon through disuse, providing information pertinent to clinical practice and relevant applications, such as plaster placement and proper support in wheelchair design.

SET is potentially indicated to be used as an initial medical screening to evaluate the presence of NED and may also serve to instruct additional, more specific, and complex examinations (27). As far as we know, we are the first study to investigate the prevalence of NED using the SET tool in individuals with chronic SCI participants. The RF and VL muscles present more marked development of NED (33.3%), followed by VM and TA muscles, with 26.6%. Notwithstanding, no significant differences were found in TA and BB muscles. Silva et al. (27) showed a higher prevalence of NED in TA in critically-ill individuals. Likewise, Fernandes et al. (28) demonstrated that the chronaxie increased in neurorrhaphy of the ulnar nerve that could be related to a negative response to the treatment and/or indicate a decline in functional condition. The discrepancy between our results compared to Silva et al. (27) and Fernandes et al. (28) could be related to the different disease and their time-course assessment between trials.

NMES has been used to stimulate paralyzed skeletal muscle in people with SCI. The primary purpose of NMES has been to reverse losses in skeletal muscle mass and improve functionality in people with incomplete paralysis (52-55). Excitability changes may not only impair nerve conduction, such as NMES clinical-like protocols. Recently, Arpin et al. (55) suggest that wide pulse widths (500 and 1,000 μs) would benefit for unresponsive SCI subjects when compared to conventional NMES protocols (short pulse widths). It seems that wide pulse widths cause greater central activation in the recruitment of more motor units without alteration of the average conduction velocity of muscle fibers Arpin et al. (55). Likewise, Guimarães et al. (56) also recommended that non-responsivity to NMES may not only be associated with muscle atrophy and motor nerve dysfunctions due to a long time of injury, but also to damages to descending motor fibers of motor neurons located on the motor cortex (disconnection syndrome). Non-responsive individuals to NMES may present damage to peripheral motor routes – in other words, located on the nerve roots and/or on the motor neuron axons (56).

Interestingly, there are several reports in the literature of non-responder individuals to NMES, which is considered a gold standard intervention to induce substantial gains in muscle mass for people with SCI (53-55). We suggest that these changes in excitability may be related not only to changes in musculotendinous architecture but also to

unresponsiveness in this population. Some of the excitability changes obtained from SCI individuals may suggest that a degree of axonal depolarization was present in their peripheral nerves (22). Several mechanisms are involved in the injury and secondary processes, which may have contributed to such changes, including ischemia (acute and chronic) and abnormal ionic shifts across cell membranes (22). Future studies should be conducted with more personalized and specific treatments, such as chronaxie-based NMES in SCI individuals (28).

Some limitations should be addressed in our study. This was a single-center study with SCI participants; thus, the findings may not be generalizable to different settings and SCI patients. Also, it was not assessed muscle atrophy with the cross-sectional area measurement. Another limitation of the study is that the tendon length was not measured, which may be altered in individuals with alterations in the central nervous system (46). The present results may under-estimated muscle atrophy, as recently described during sepsis (57). However, despite the higher sensitivity of the cross-sectional area compared with thickness, we were able to detect significant statistical differences with excellent reliability. Also, it was not evaluated the mechanical and material tendon proprieties which limit the comparison amongst trials. Future studies with SET should be developed in order to investigate the presence of NED using larger samples size, using major clinical outcomes in other clinical populations.

In conclusion, muscles and tendons maladaptation under the lesion level occurs globally in individuals with chronic SCI. The increased chronaxie values and the higher NED prevalence for SCI seem to play a critical role in the maladaptation of muscle and tendon architecture, revealing a possible acquired peripheral neuropathy.

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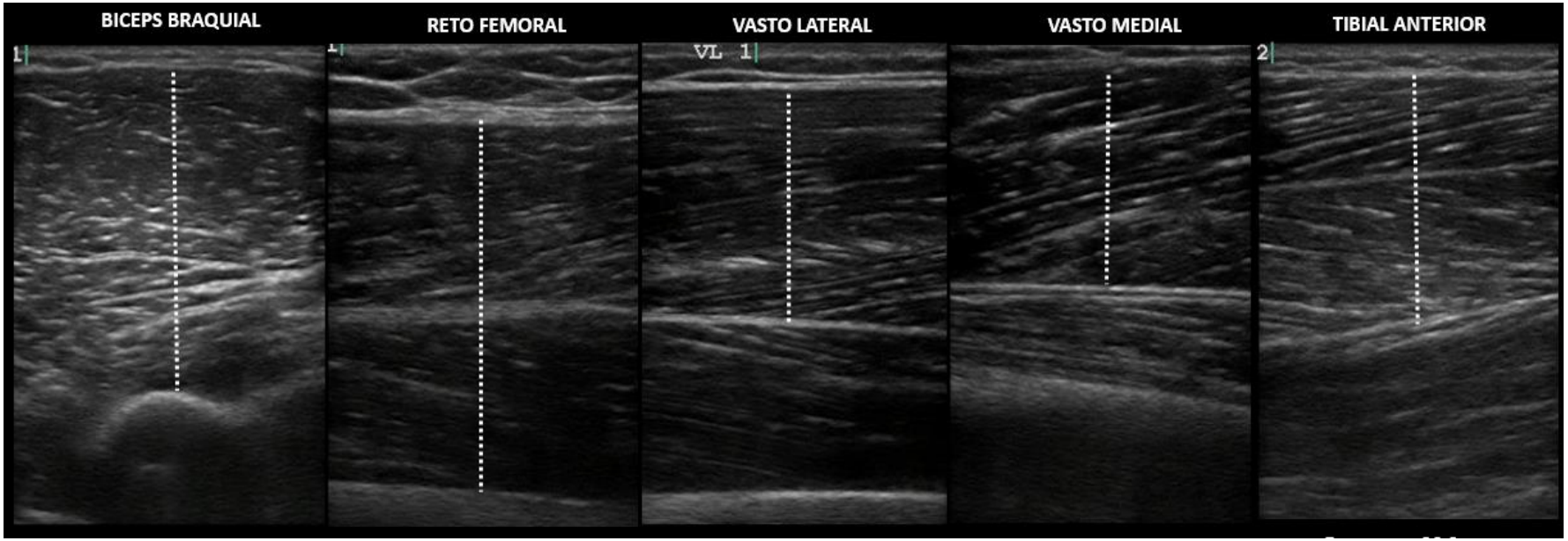
A.



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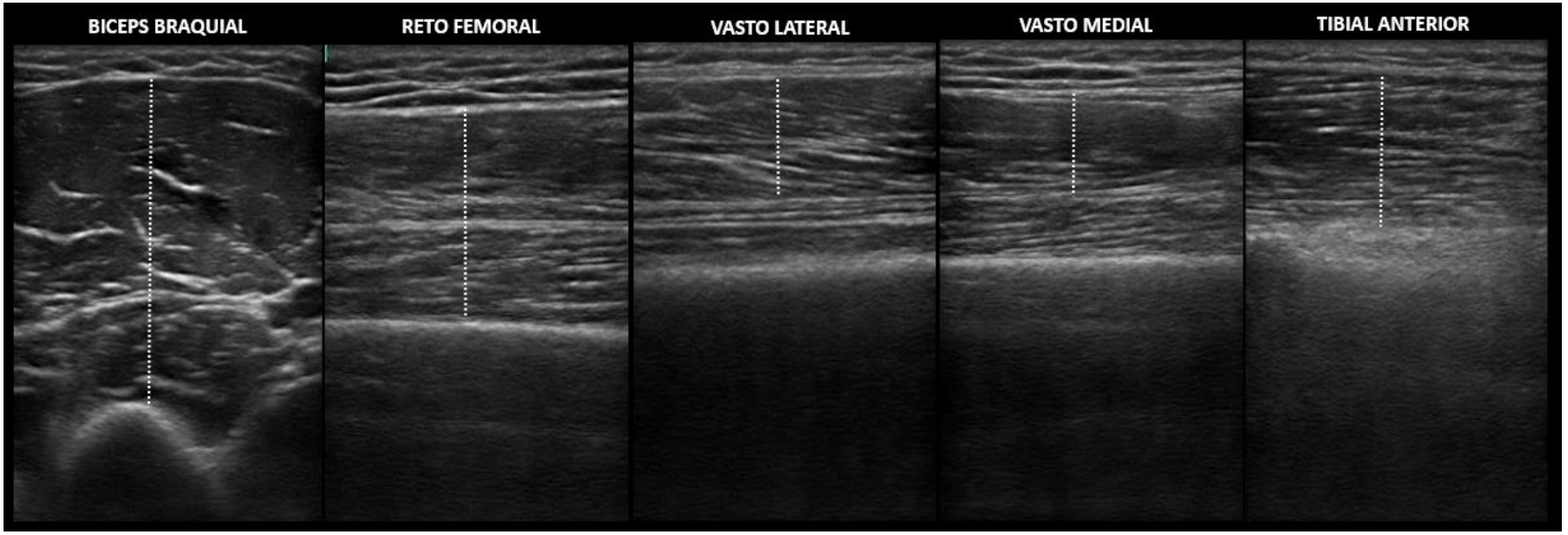

Figure 1. Muscle thickness measurement sites in the able-bodied group (A) and in the spinal cord injury group (B), in the biceps brachii muscles; rectus femoris; vastus lateralis, vastus medialis and anterior tibialis after image acquisition.

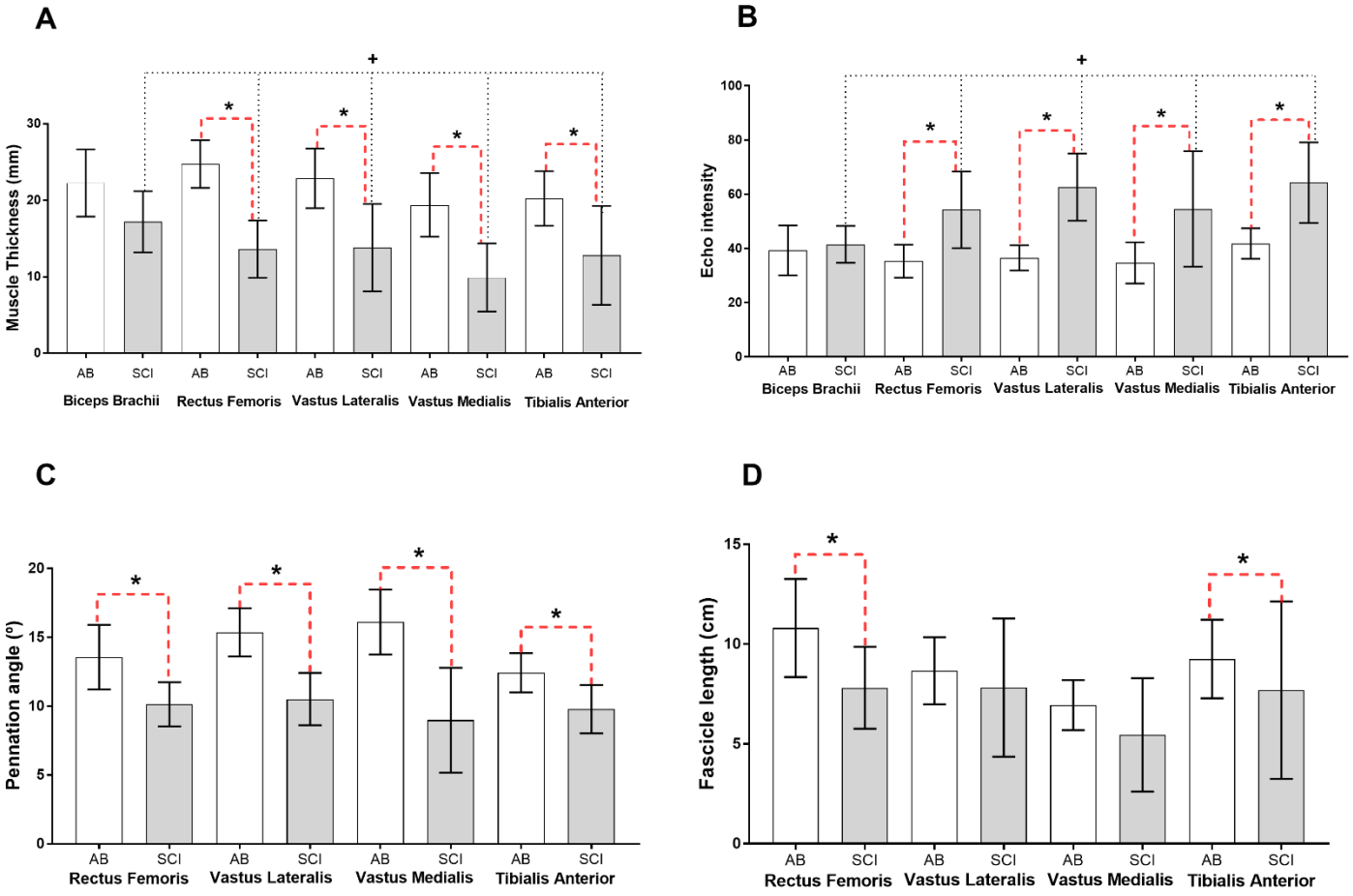

Figure 2. Bar graphs comparing muscle ultrasonography variables: thickness (A), echo intensity (B), pennation angle (C), and fascicle length (D), obtained in the BB, RF, VL, VM, and TA, in SCI and Able-bodied. The difference in BB in the SCI from the other SCI muscles ($p < 0.05$) is represented by the plus sign (+). Red dotted loops indicate comparisons between muscle groups between SCI and able-bodied subjects. Significant differences ($p < 0.05$) are represented by an asterisk (*). SCI = Spinal Cord group; AB = Able-bodied group; BB= biceps brachii; RF = rectus femoris; VL = vastus lateralis; VM = vastus medialis; TA = anterior tibialis.

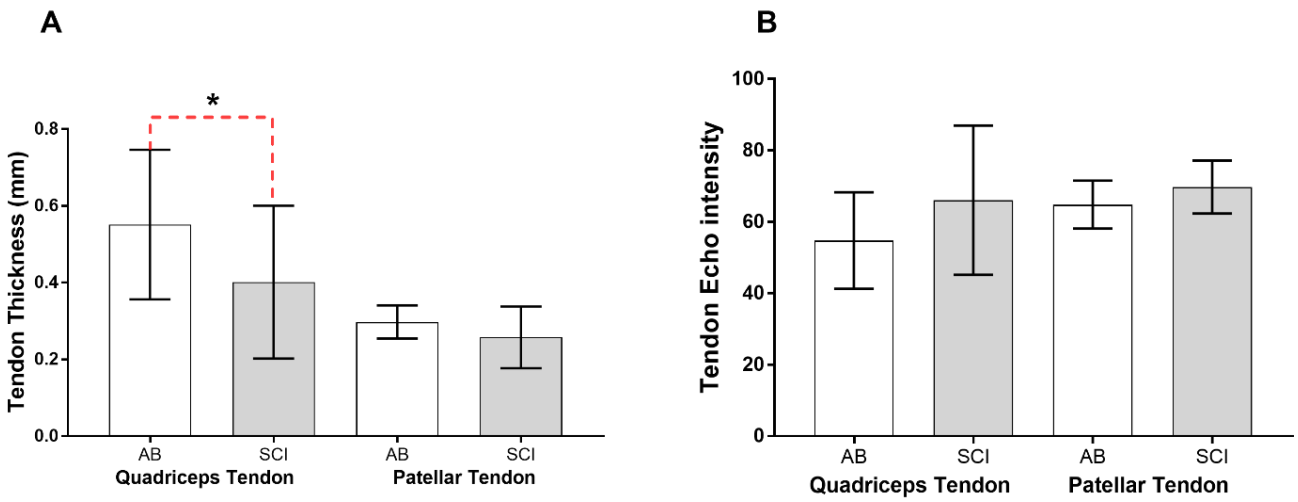

Figure 4. Bar graphs comparing the tendon ultrasound variables: thickness (A), echo intensity (B), obtained in the quadriceps and patellar tendons, in the SCI and able-bodied groups. Red dotted loops indicate comparisons between SCI and Able-bodied. Significant differences ($p < 0.05$) are represented by an asterisk (*). SCI = Spinal Cord group; AB = Able-bodied group.

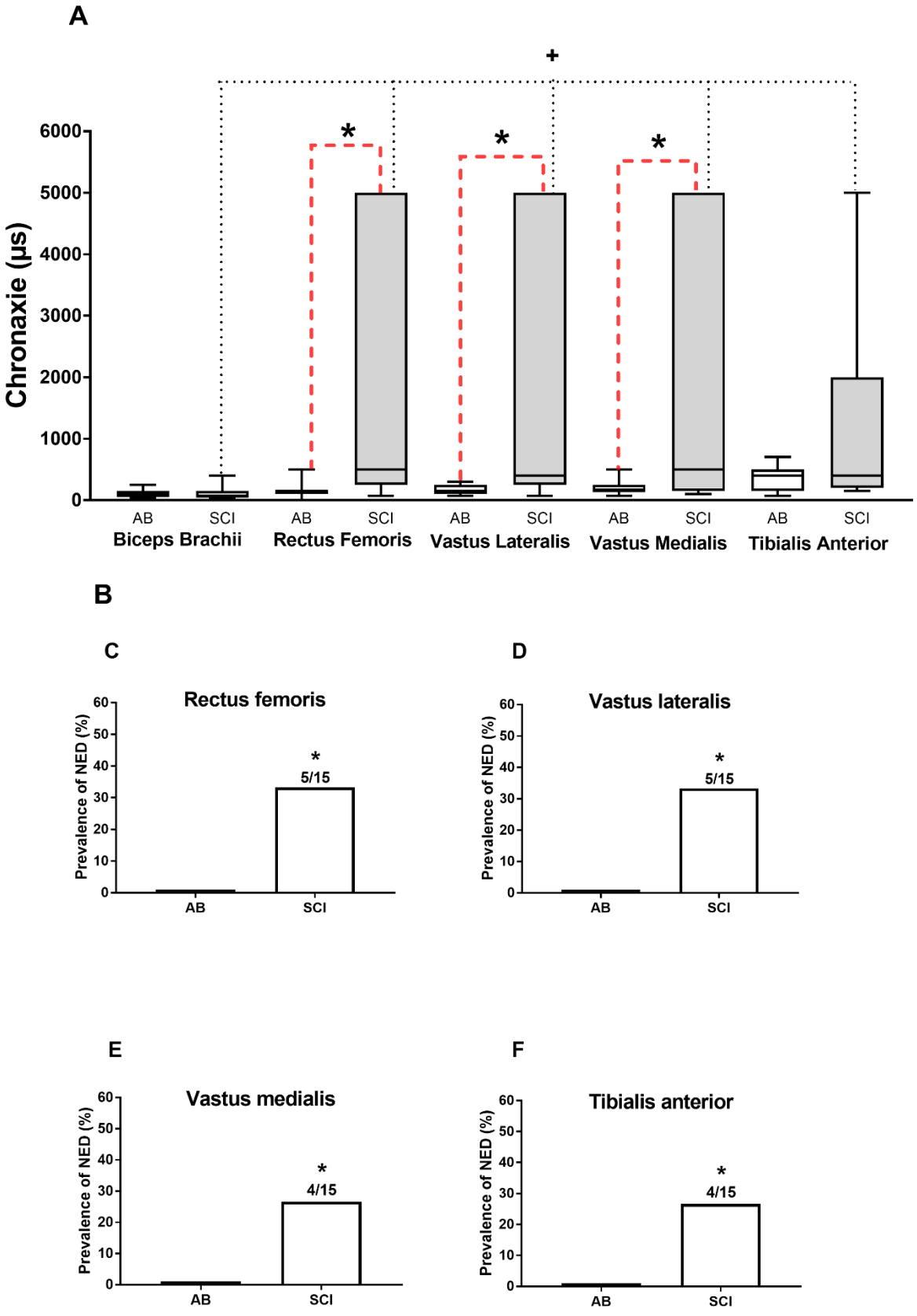

Figure 5. A. Boxplot graphs comparing the chronaxis (µs) obtained in the BB, RF, VL, VM, and TA muscles, in the SCI and able-bodied groups. B. Prevalence of neuromuscular electrophysiological disorders (NED) in the RF (C), VL (D), VM (E), and TA (F) muscles in the SCI and able-bodied groups. The difference in BB in the SC from the other SCI muscles ($p < 0.05$) is represented by the plus sign (+). Red dotted loops indicate comparisons between SCI and Able-bodied muscles. Significant differences ($p < 0.05$) are represented by an asterisk (*). SCI = Spinal Cord group; AB = Able-bodied group.

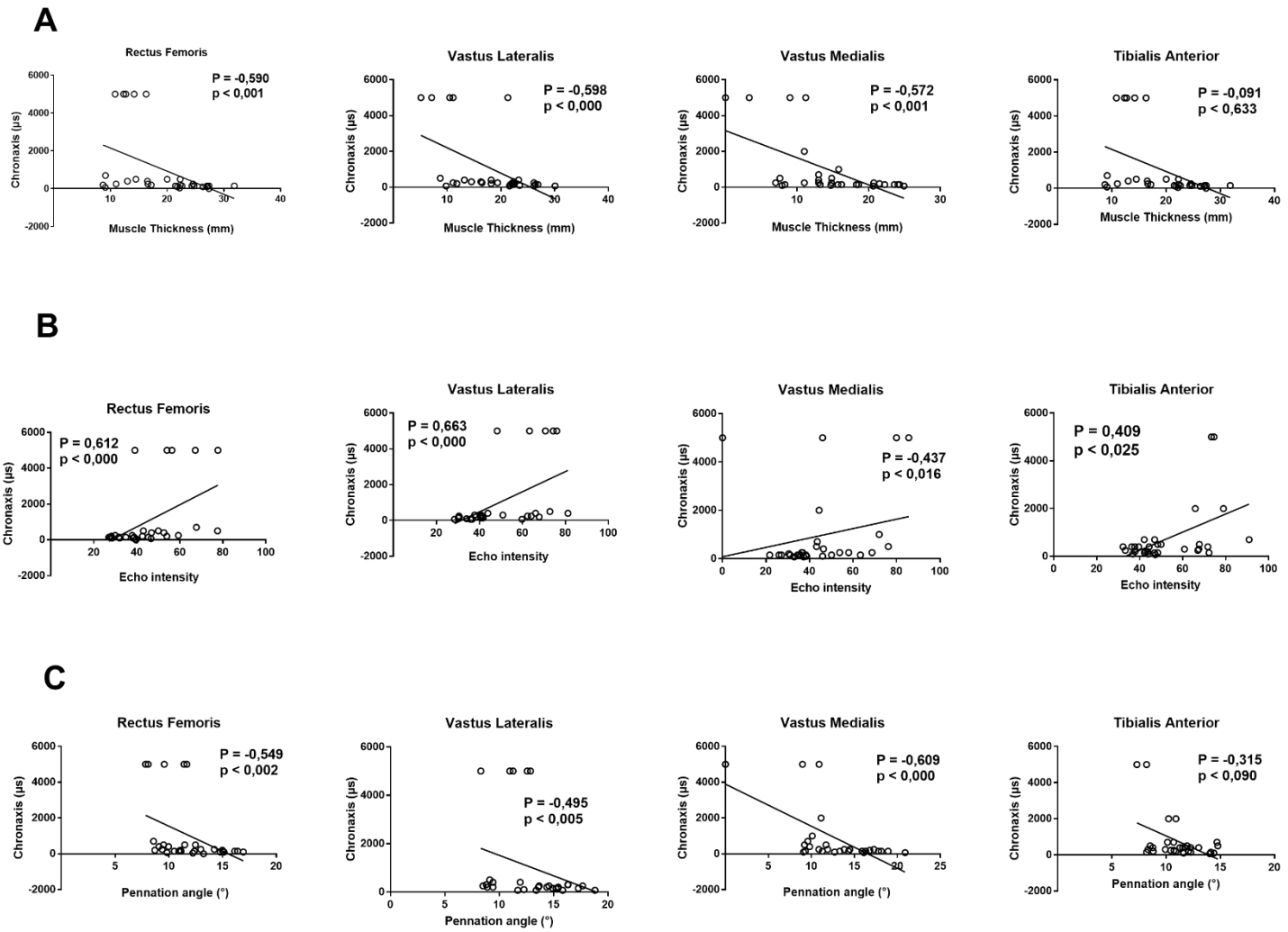

Figure 6. A. Correlation graphs between chronaxis and muscle thickness in RF, VL, VM and TA; B. Correlation between chronaxis and muscle echogenicity in RF, VL, VM and TA; C. Correlation between chronaxis and penetration angle in RF, VL, VM and TA. BB= biceps brachii; RF = rectus femoris; VL = vastus lateralis; VM = vastus medialis; TA = anterior tibialis.

4. DISCUSSÃO GERAL

Os principais achados deste estudo estão de acordo com a hipótese inicial de que a presença de atrofia muscular crônica em indivíduos com LM promove alterações na arquitetura muscular, observadas através da redução da espessura, aumento da ecogenicidade e diminuição do ângulo de penação e comprimento fascicular, associado a alterações na excitabilidade neuromuscular relacionada à presença de NED. O SET associado a ultrassonografia muscular e tendínea, são recursos que podem ser utilizados para auxiliar no diagnóstico, avaliar o estágio de uma lesão nervosa e o seu comprometimento a curto e longo prazo, além de auxiliar na escolha dos parâmetros utilizados na eletroestimulação.

Neste estudo, foi demonstrado que os participantes do grupo LM apresentaram menores valores de espessura, ângulo de flexão e comprimento fascicular e maiores valores de ecogenicidade quando comparados aos participantes saudáveis. Foram encontrados valores significativamente mais baixos na espessura nos músculos RF, VL, VM e TA (redução de quase 40% em comparação com os músculos saudáveis). Resultados semelhantes foram observados em estudos que utilizaram técnicas de ressonância magnética em indivíduos com LM (Shah et al., 2006; Giangregorio et al., 2006; Smith et al., 2017). Além disso, há um interesse crescente na mensuração de alterações na qualidade muscular (ecogenicidade), particularmente no processo de desuso muscular. Um aumento na ecogenicidade, como visto anteriormente neste trabalho, resulta em músculo mais branco ou brilhante (hiperecoico) (Reimers et al., 1993; Berry et al., 2019). É possível que o aumento da ecogenicidade esteja relacionado ao tipo de envolvimento musculotendíneo ao qual os indivíduos com LM estão expostos, como imobilidade prolongada, hipotrofia ou atrofia causada por desuso, resposta inflamatória sistêmica, deposição de gordura na musculatura e reposição de tecido muscular por fibrose (Reimers et al., 1993; Parry et al., 2015).

No presente estudo, a perda de espessura muscular foi correlacionada com uma redução significativa no ângulo de penação da musculatura fascicular e comprimento do fascículo. Em relação ao ângulo de penação, nossos resultados mostraram valores mais baixos nos músculos RF, VL, VM e TA, quando comparados ao grupo pareado. Não há evidências de que os indivíduos com LM apresentem fascículos musculares ou ângulo de penação menor. Alguns estudos, como o de Morse et al. revelaram uma deterioração de 12% no ângulo de penação do músculo gastrocnêmico em indivíduos mais velhos (Morse

et al., 2005). Além disso, Turton et al. demonstraram uma diminuição no ângulo de penação do VL, após 5-10 dias de imobilidade prolongada em unidade de terapia intensiva com uso de ventilação mecânica invasiva (Turton et al., 2019). Diong et al. demonstrou que o ângulo de penação e comprimento do fascículo no músculo gastrocnêmio foi significativamente menor na LM do que os indivíduos controle, corroborando com o presente estudo (Diong et al., 2012). Esses achados são consistentes com outros dados que mostram que a atrofia causa uma redução na penação (Morse et al., 2005; Turton et al., 2019). Curiosamente, o ângulo de penação está relacionado à capacidade de armazenar mais componentes contráteis em um determinado volume e pode gerar maior força com ângulos de até 45 graus (Aagaard, 2001). Se a perda no ângulo de torção levar à deterioração na geração de força, isso pode ajudar a explicar a fraqueza muscular associada a modificações funcionais devido ao nível de lesão em indivíduos com LM. Além disso, demonstramos que nos músculos RF, VL, VM e TA, a espessura muscular e o ângulo de penação estão relacionados e apoiam estudos anteriores que afirmaram que músculos com uma espessura maior poderiam gerar mais força devido a seus maiores ângulos de penação e, consequentemente, maiores áreas transversais (Aagaard, 2001; Turton et al., 2019). Uma vez que os ângulos de penação estão diretamente relacionados às propriedades de geração de força (Lieber e Fridén, 2000; Sopher et al., 2017), nossos resultados sugerem que o ângulo de penação deve ser monitorado em indivíduos com LM.

Embora as adaptações musculares após LM (atrofia) tenham sido extensivamente estudadas (Castro et al., 1999; Shah et al., 2006; Kern et al., 2017; Thomaz et al., 2019), o remodelamento do tendão permanece amplamente desconhecido nessa população. Dudley-Javoroski (2010) e col. avaliaram o tendão patelar e o tendão de Aquiles em indivíduos com LM, mas não detectou diferenças na espessura do tendão (Dudley-Javoroski et al., 2010). Apenas no estudo de Maganaris et al. (2006) foi relatado que indivíduos com LM crônica apresentaram uma área de secção transversal do tendão patelar 17% menor do que indivíduos saudáveis. É possível sugerir que esses tendões se alongam com o uso prolongado de cadeira de rodas, devido ao posicionamento que mantém o joelho flexionado continuamente a 90 graus (Maganaris et al., 2006). Pesquisas adicionais são necessárias para elucidar ainda mais o papel da posição articular na deterioração do tendão humano por desuso, fornecendo informações diretamente pertinentes à prática clínica e aplicações relevantes (Maganaris et al., 2006).

A deterioração na arquitetura do tendão, como resultado da paralisia, não pôde ser identificada no presente estudo, bem como em Maganaris e colaboradores, 2006. O tendão é um tecido metabolicamente ativo, como osso e músculo, adapta-se a cargas mecânica imposta (Dudley-Javoroski et al., 2010). Estudos com atletas saudáveis (Brumitt e Cuddeford et al., 2015) mostraram uma área transversa do tendão maior do que em controles não treinados, sugerindo que a exposição crônica ao exercício pode melhorar a arquitetura do tendão (Dudley-Javoroski et al., 2010). As atividades de treinamento muscular induzem a síntese proteica de colágeno, no entanto, a carga tênsil insuficiente gera um desequilíbrio na matriz extracelular, especialmente no colágeno, levando à má adaptação do tendão (Maganaris et al., 2017). Dessa forma, pesquisas futuras envolvendo eletroestimulação muscular por um longo período podem sugerir alterações tendíneas mais benéficas.

O SET é potencialmente indicado para ser usado como uma triagem inicial para avaliar a presença de NED e também pode servir para instruir exames adicionais, mais específicos e complexos (Silva et al., 2018). Até onde sabemos, este trabalho é o primeiro a investigar a prevalência de NED usando a ferramenta SET em indivíduos com lesão medular crônica. Os músculos RF e VL apresentam desenvolvimento mais acentuado de NED (33,3%), seguido pelos músculos VM e TA com 26,6%. Apesar disso, não foram encontradas diferenças significativas nos músculos TA e BB. Apesar de não haver estatística significante de NED no TA e no BB, foi observado um aumento da cronaxia mais específico para o TA (cronaxia atingindo 1170 μs, em média). Esse é um achado relevante que pode afetar diretamente o tratamento e os resultados funcionais de pacientes gravemente enfermos. Por outro lado, Silva et al. (2018) mostraram uma maior prevalência de NED na TA em indivíduos gravemente enfermos. Além disso, Fernandes et al. (2016) demonstraram que a cronaxia pode ser usada para monitorar a regeneração neural e que alterações na cronaxia aumentou a neurorráfia do nervo ulnar que poderia estar relacionada à resposta negativa ao tratamento e/ou indicar um declínio na condição funcional. A discrepância entre os resultados encontrados nesse estudo em comparação com Silva et al. (2018) e Fernandes et al. (2016) podem estar relacionados às diferentes doenças e seu tempo avaliado entre os ensaios e o tempo dessas doenças. É essencial estabelecer quais músculos e nervos são mais afetados e quando ocorrem distúrbios após lesão medular. Assim, tratamentos mais personalizados e específicos, como o NMES à base de cronaxia, podem ser recomendados para pacientes com NED (Fernandes et al., 2016).

Os parâmetros de reobase e cronaxia foram estudados por Lee et al. em pacientes que sofrem de encefalopatia após acidente vascular cerebral. Os resultados obtidos para os lados parético e não parético foram comparados, mostrando que os valores de reobase e cronaxia foram significativamente maiores para o lado parético, o que corrobora o presente estudo. Pode-se inferir que a redução da atividade muscular em casos de paresia, paralisia e lesão de nervo periférico contribuíram para a necessidade de maior estímulo em termos de intensidade e duração (Fernandes et al., 2016).

O NMES tem sido usado para estimular o músculo esquelético paralisado em pessoas com LM. O objetivo principal do NMES é reverter as perdas na massa muscular esquelética e melhorar a funcionalidade em pessoas com paralisia incompleta (Bickel et al., 2015 Bochkezanian et al., 2018; de Freitas et al., 2018; Arpin et al., 2019). No entanto, existem vários relatos na literatura de indivíduos que não respondem ao NMES, que é considerada uma intervenção padrão-ouro para induzir ganhos substanciais na massa muscular de pessoas com LM (de Freitas et al., 2018; Bochkezanian et al., 2018 Arpin et al., 2019). Sugerimos que essas mudanças na excitabilidade podem estar relacionadas não apenas às mudanças na arquitetura musculotendinea, mas também à não responsividade dos indivíduos com LM a eletroestimulação convencional. Algumas das mudanças de excitabilidade obtidas de indivíduos com LM podem sugerir que um grau de despolarização axonal esteja presente em seus nervos periféricos (Shin-Yi Lin et al., 2007). Vários mecanismos estão envolvidos na lesão e nos processos secundários que podem ter contribuído para essas alterações, incluindo isquemia (aguda e crônica) e alterações iônicas anormais nas membranas celulares (Shin-Yi Lin et al., 2007). Os mecanismos por trás da ativação central e da não responsividade promovidos pelo NMES não são bem compreendidos. Estudos futuros devem ser conduzidos com tratamentos mais personalizados e específicos, como NMES à base de cronaxia em indivíduos com LM (Fernandes et al., 2016).

As medidas de espessura muscular do RF, VL e VM se correlacionaram negativamente com a cronaxia, demonstrando que quanto maior a cronaxia menor a espessura muscular. Para ecogenicidade muscular foram encontradas correlações positivas nos músculos RF, VL e TA, demostrando que quanto maior a cronaxia, maior a ecogenicidade. No ângulo de penação, foram encontradas correlações negativas para os músculos RF, VM e TA demostrando que quanto maior a cronaxia, menor o ângulo de penação muscular. Pro comprimento fascicular não forma encontradas correlações

significativas. Futuro estudos devem ser realizados para elucidar melhor essas correlações e o seu impacto na pratica clínica.

Em termos de aplicações clínicas, os resultados deste estudo reforçam a necessidade de uma avaliação detalhada, quantitativa e cuidadosamente direcionada de indivíduos com lesão medular crônica. Os resultados também indicam a conveniência de restabelecer uma avaliação eletrodiagnóstica associada às variáveis ultrassonográficas na prática clínica. A cronaxia, especialmente, é um parâmetro valioso que pode ser usado em avaliações durante o processo de recuperação. O valor da cronaxia é benéfico para determinar a duração do impulso elétrico utilizado para estimular o músculo e auxiliar na aplicação de um estímulo mais confortável. A duração ideal de um pulso é igual à cronaxia muscular que ele visa estimular (Spielhoz, 2002). Como o teste de cronaxia requer o valor de reobase, também temos uma indicação de um possível valor de intensidade de estimulação (Fernandes et al., 2016). Coletivamente, os resultados apresentados aqui podem ajudar as equipes de reabilitação a projetar protocolos de estimulação personalizados para otimizar a aplicação clínica da terapia de eletroestimulação.

Vários fatores limitam este estudo, à heterogeneidade dos indivíduos com LM em relação ao nível e tipo de lesão medular. Outra limitação desse estudo foi a não realização de testes biomecânicos para avaliar a função muscular e tendínea. Estudos futuros com SET devem ser desenvolvidos para investigar a presença de NED usando amostras maiores e em outras populações.

5. CONCLUSÃO

Em conclusão, a atrofia por desuso ocorre globalmente em indivíduos com LM crônica abaixo do nível da lesão, reduzindo a espessura muscular, o ângulo de penação e o comprimento fascicular e aumentando a ecogenicidade dos músculos e tendões. Resultados importantes foram encontradas nesse estudo, inferindo que um aumento nos valores de cronaxias e a presença de NED poderia ser um parâmetro relacionado as alterações na arquitetura musculotendínea.

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7. ANEXOS

Anexo I – Documento de aprovação do projeto por Comitê de Ética em Pesquisa

UNB - FACULDADE DE CEILÂNDIA DA UNIVERSIDADE DE BRASÍLIA

Continuação do Parecer: 2.513.746

pesquisadores associados e ao pessoal técnico integrante do projeto; e justificar fundamentadamente, perante o CEP ou a CONEP, interrupção do projeto ou a não publicação dos resultados.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1048573.pdf	30/01/2018 13:06:40		Aceito
Recurso Anexado pelo Pesquisador	carta_para_encaminhamento_de_pendencias.pdf	30/01/2018 13:06:03	LARISSA VIEIRA SANTANA	Aceito
Outros	curriculo_lattes_joao.pdf	30/01/2018 13:03:23	LARISSA VIEIRA SANTANA	Aceito
Outros	curriculo_lattes_Jonathan.pdf	30/01/2018 13:03:02	LARISSA VIEIRA SANTANA	Aceito
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Projeto Detalhado / Brochura Investigador	Projeto_completo.pdf	30/01/2018 12:58:11	LARISSA VIEIRA SANTANA	Aceito
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