UNIVERSIDADE DE BRASÍLIA-UnB
FACULDADE DE CEILÂNDIA-FCE
CURSO DE FISIOTERAPIA

ÁLVARO DE ALMEIDA VENTURA
LEANDRO GOMES DE JESUS FERREIRA

DOES ELECTRODES PLACEMENT OVER THE MOTOR POINT OF VASTUS LATERALIS AND MEDIALIS AFFECT DIFFERENT PORTIONS OF QUADRICEPS MUSCLE ARCHITECTURE DURING SUBMAXIMAL EVOKED TORQUE? A SECONDARY ANALYSIS OF A RANDOMIZED CROSS-OVER TRIAL

BRASÍLIA
2021

ÁLVARO DE ALMEIDA VENTURA
LEANDRO GOMES DE JESUS FERREIRA

DOES ELECTRODES PLACEMENT OVER THE MOTOR POINT OF VASTUS LATERALIS AND MEDIALIS AFFECT DIFFERENT PORTIONS OF QUADRICEPS MUSCLE ARCHITECTURE DURING SUBMAXIMAL EVOKED TORQUE? A SECONDARY ANALYSIS OF A RANDOMIZED CROSS-OVER TRIAL

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.
Orientador (a): Prof. Dr. João Luiz Quagliotti Durigan
Coorientador (a): Prof. Me. Jonathan Galvão Tenório Cavalcante

BRASÍLIA
2021

ÁLVARO DE ALMEIDA VENTURA
LEANDRO GOMES DE JESUS FERREIRA

DOES ELECTRODES PLACEMENT OVER THE MOTOR POINT OF VASTUS LATERALIS AND MEDIALIS AFFECT DIFFERENT PORTIONS OF QUADRICEPS MUSCLE ARCHITECTURE DURING SUBMAXIMAL EVOKED TORQUE? A SECONDARY ANALYSIS OF A RANDOMIZED CROSS-OVER TRIAL

Brasília, 04/11/2021

COMISSÃO EXAMINADORA

_____

Prof. Dr. João Luiz Quagliotti Durigan
Faculdade de Ceilândia - Universidade de Brasília-UnB
Orientador

_____

Prof.ª Drª. Patrícia Azevedo Garcia
Faculdade de Ceilândia - Universidade de Brasília-UnB

_____

Prof. Dr. Wagner Rodrigues Martins
Faculdade de Ceilândia - Universidade de Brasília-UnB

Dedicatória

Este trabalho é dedicado aos nossos pais, familiares, amigos e professores por todo apoio e incentivo.

AGRADECIMENTOS

A Deus por ter me dado a oportunidade de nascer numa família que sempre teve condições de me proporcionar uma boa educação, o que me foi crucial para chegar até aqui. A minha família, em especial aos meus pais, que foram os responsáveis pela minha vida, por ter cuidado de mim e que guiaram meus passos até eu chegar a Universidade. Ao meu irmão e à minha namorada por todo apoio ao longo do caminho e por terem sido minhas fiéis cobaias para as provas práticas. A Universidade de Brasília e a FAP-DF, por ter me proporcionado grandes oportunidades de ensino e principalmente de pesquisa. Aos professores que cruzaram o meu caminho e que de alguma forma me deixaram algum conhecimento e alguma memória, em especial a Professora Jamila Reis e Luis Muehlman, por terem despertado em mim o prazer em estudar e aprender novas coisas no início da graduação; ao Professor Emerson Fachin, por ter me dado a primeira oportunidade como monitor e como bolsista; a Professora Patrícia Garcia, pela confiança em todas as muitas monitorias juntos; ao Professor Osmair Gomes, pela paciência em ensinar e pelas nossas conversas sempre esclarecedoras durante as monitorias; ao Professor Wagner Martins pela parceria e amizade construída e por último ao Professor João Durigan, pela confiança em aceitar a mim e ao Léo como alunos de Iniciação Científica quando tudo ainda era muito incerto, pelas oportunidades que tem nos dado até agora, por aceitar ser nosso Orientador durante a graduação e pela amizade que vem se construindo. Gostaria também de agradecer aos meus colegas, por todas as aulas, trabalhos, provas e dias estudando que fizeram parte de todo o aprendizado construído até aqui, que sejam todos realizados com essa linda profissão! E como não poderia deixar de ser, um último e sincero agradecimento mais que especial ao meu irmão de coração e fiel escudeiro, Leandro Gomes, por toda a parceria, pela amizade e por todo esse caminho que construímos juntos até aqui. Desde o primeiro dia, entramos juntos nessa faculdade e até hoje estamos caminhando juntos. Participando dos mesmos projetos, das mesmas turmas, das mesmas matérias, mesmos grupos de trabalho e etc, pela certeza de poder confiar um ao outro. Sozinhos podemos ir mais rápido, mas juntos nós vamos mais longe, e sem você eu poderia até me formar, mas tenho certeza que não teria construído uma história tão bonita quanto a nossa foi durante a graduação. Obrigado a todos!

ÁLVARO VENTURA

Gostaria de agradecer a Deus por ter trilhado meu caminho e me permitido estudar em uma Universidade sólida, renomada e acolhedora que é a Universidade de Brasília (UNB). Sou grato a todo corpo docente que de alguma forma contribuiu para formar o profissional que sou hoje. Em especial gostaria de agradecer à Professora Patrícia Azevedo Garcia por ter despertado meu interesse na área da Fisioterapia Ortopédica, e por ter confiado em mim como monitor em suas disciplinas. Ao Professor João Paulo Chieregato Matheus, por todas as conversas sobre futuro profissional e esclarecimentos sobre a Fisioterapia. Ao Professor Osmair Gomes por ser um exemplo de profissional e ter uma didática muito boa para ensinar seus alunos. Ao professor Wagner Rodrigues Martins por ter sido meu primeiro professor do estágio, no Hospital de Base, por toda parceria criada, que foi um divisor de águas na minha vida como futuro fisioterapeuta. Ao meu amigo Jonathan Galvão, que me ajudou na minha primeira iniciação científica e que contribuiu maravilhosamente neste trabalho. Por último, meus profundos agradecimentos ao Professor João Durigan por ter me aceitado como aluno de iniciação científica, por ter introduzido a importância da pesquisa na minha carreira profissional, por ter aceitado ser meu orientador nesse trabalho mais que especial, por todas as conversas e pela amizade que criamos. Além disso, agradeço à Universidade de Brasília e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo incentivo à pesquisa e apoio financeiro fornecido para o desenvolvimento dos meus projetos.

Agradeço aos meus pais, Francineide e Getúlio, e à minha avó, Terezinha, que me mostraram como ser uma pessoa de caráter, que me incentivaram desde criança a ter gosto pelo estudo, além de terem fornecido as melhores oportunidades para que isso acontecesse. À minha namorada, Gabriele, por ser uma das minhas maiores incentivadoras, por acreditar no meu potencial e por me mostrar que por mais que as coisas pareçam difíceis eu posso ser sempre mais forte e passar por cima de qualquer obstáculo. Aos meus amigos por tornarem essa jornada mais leve, sem vocês não seria tão divertido.

Em especial, gostaria de agradecer ao meu irmão Álvaro Ventura por ser meu maior parceiro nessa caminhada, por ter topado junto a mim fazer parte de vários cursos, projetos, monitorias, disciplinas e noites mal dormidas. Que prazer imenso foi ter encontrado nessa amizade uma pessoa que só agrega coisas boas em minha vida, sem você teria sido muito mais difícil, e acredito que não seria a pessoa que eu amo ser hoje. Fique sabendo que você é meu exemplo, obrigado por me incentivar a estudar e treinar todos os dias, com toda certeza juntos somos mais fortes e vamos construir um futuro brilhante pela frente. Obrigado!

LEANDRO GOMES

“As vezes a felicidade demora a chegar

Aí é que a gente não pode deixar de sonhar

Guerreiro não foge da luta não pode correr

Ninguém vai poder atrasar quem nasceu pra vencer”

(Grupo Revelação)

ABSTRACT

Background: Few studies has evaluated possible differences in muscle architecture in quadriceps femoris constituents when electrodes are positioned directly over vastus lateralis (VL) and vastus medialis (VM) motor points during a neuromuscular electrical stimulation (NMES) session. **Objectives:** To investigate whether there is a difference in the behavior of the rectus femoris (RF), VL, VM and vastus intermedius (VI) during an evoked contraction with the electrodes placed over the motor point of the VL and VM. **Methods:** This is a sub-study of a larger controlled trial, conducted with young, healthy male subjects who underwent contractions with NMES on quadriceps femoris muscle. The observed variables were: pennation angle (θ_p) and fascicle length (L_f) of VL, VM, VI, RF (at rest and in evoked contraction) evaluated by ultrassonography, and torque at 40% of maximum voluntary contraction synchronizing the torque tracing with the ultrasonographic recordings. **Results:** There was no difference for θ_p comparing four components of quadriceps femoris ($F_{3.57} = 1.33$, $p = 0.27$, $\eta\rho 2$: 0.06, power: 0.33). And for L_f, ANOVA showed a significant interaction for muscle x contraction ($F_{3.57} = 3.17$, $p < 0.05$, $\eta\rho 2$: 0.14, power: 0.70). The Tukey post-hoc indicated only interactions not relevant to the study though. **Conclusion:** There are no acute differences in the behavior of the components of the quadriceps femoris during stimulation with the electrodes placed on the motor points of the VL and VM.

Keywords: Neuromuscular electrical stimulation; ultrasonography; muscle architecture; electrodes placement; quadriceps femoris; isokinetic dynamometer.

RESUMO

Background: Poucos estudos avaliaram as possíveis diferenças na arquitetura muscular dos componentes do quadríceps femoral quando os eletrodos são posicionados diretamente sobre os pontos motores do vasto lateral (VL) e vasto medial (VM) durante uma sessão de eletroestimulação neuromuscular (NMES). **Objetivos:** Investigar se há diferença no comportamento do reto femoral (RF), VL, VM e vasto intermédio (VI) durante contrações evocadas com eletrodos posicionados sobre os pontos motores do VL e VM. **Métodos:** Trata-se de um estudo secundário de um ensaio clinico com homens jovens e saudáveis que foram submetidos a contrações com NMES sobre o quadríceps femoral foi conduzido. As variáveis observadas foram: ângulo de penação (θ_p) e comprimento do fascículo (L_f) do VL, VM, VI, RF (em repouso e contração evocada) avaliadas por ultrassonografia, e torque a 40% da contração voluntária máxima que foi sincronizado com as medidas ultrassonográficas. **Resultados:** Não houve diferença para o θ_p quando comparado os quatro componentes do quadríceps femoral ($F3.\ 57 = 1.33$, $p = 0.27$, $\eta\rho2$: 0.06, power: 0.33). E para o L_f, a ANOVA mostrou uma interação significativa para músculo x contração ($F3.\ 57 = 3.17$, $p < 0.05$, $\eta\rho2$: 0.14, power: 0.70). O Tukey post-hoc indicou apenas interações não relevantes para nosso estudo. **Conclusão:** Não houve diferenças agudas no comportamento dos componentes do quadríceps femoral durante eletro estimulação com os eletrodos colocados sobre os pontos motores do VL e VM.

Palavras-chave: Estimulação elétrica neuromuscular; ultrassonografia; arquitetura muscular; posicionamento de eletrodos; quadríceps femoral; dinamômetro isocinético.

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

ACL: Anterior cruciate ligament

IPAQ: International Physical Activity Questionnaire

L_f: Fascicle length

MEIC: Maximum evoked isometric contractions

MVC: Maximal voluntary contraction

NMES: Neuromuscular electrical stimulation

θ_p: Pennation angle

RF: Rectus femoris

SD: Standard deviation

VI: Vastus intermedius

VL: Vastus lateralis

VM: Vastus medialis

SUMMARY

1- INTRODUCTION

Neuromuscular electrical stimulation (NMES) is a tool used in clinical practice to promote the activation of peripheral motor nerves to produce skeletal muscle contraction. NMES is used to rehabilitate immobilized patients to preserve muscle mass and function, even in Anterior Cruciate Ligament (ACL) reconstruction to restore muscle strength.[1,2] However, NMES can promote visible contractions, the nature of the evoked contraction differs from the voluntary contraction.[3] The evoked contraction occurred by synchronous recruitment of the muscle fibers, while the voluntary contraction has asynchronous recruitment. In addition, the evoked contraction seems to recruit fast fibers regarding slow muscle fibers.[4-6] Thus, it has raised interest in knowing the extent of these differences to the muscle architecture because this information can guide the prescription of NMES to specific muscle adaptations.[7]

Muscle architecture is one of the main factors that influence the force generation capacity[8-10] because it provides biomechanical characteristics that are specific to a muscle and determines its function.[9,11] The main variables of the muscle architecture are the fascicle length (L_f), and the pennation angle (θ_p), which are expected to, respectively, reduce and increase during an isometric contraction.[8,12,13] Ultrasonography is a resource that can assist in real-time visualization of muscle architecture by observing fascicular movement and deep aponeurosis *in vivo*, which allows measuring changes in θ_p and L_f during skeletal muscle contraction.[8] Ultrasound was previously used to measure muscle thickness, L_f, and θ_p in multiple regions of the four quadriceps muscles in rest to describe the complex architecture of this muscle group.[14] Despite that, muscle architecture has been little explored during NMES.

NMES evokes muscle contraction of motor axons through electrodes placed over a muscle or nerve. NMES commonly is applied through two electrodes, an "active" cathode and a "return" anode, placed on the skin over a muscle belly (or muscle group) to target the motor points.[15] On quadriceps muscle, interestingly, the electrodes are commonly positioned only over the motor points of vastus medialis (VM) and vastus lateralis (VL) during the NMES session.[16-18] A systematic review evaluated the gain of strength and function, utilizing NMES, after ACL reconstruction, and the majority of studies included have reported the electrode configuration over the vastus.[19] Also, Vieira et al.[20] showed that a larger distance between electrodes in NMES provides a greater amount of torque because the relative number of motor fibers stimulated are affected by a larger area of current, which justifies the placement on VL proximal and VM distal. However, a previous study [21] observed that electrode configuration did not seem to alter the traditional measurements of quadriceps activation, but positioning on rectus femoris (RF) presented greater torque concerning the positioning on vastus. The authors admitted that the exact mechanisms behind this finding are unclear; they speculated that greater torque production with the rectus configuration might result from greater amounts of adipose tissue in the areas where the vastus electrodes are positioned than the rectus electrodes were placed. Therefore, further studies should be done to confirm these results and justify the placement of electrodes over the RF for greater torque production. Additionally, Torry et al.[22] showed that the vastus is more affected in a situation of arthrogenic inhibition, justifying the use of NMES on these muscles in a more common way in clinical practice.

As far as we know, the muscular architecture of the quadriceps femoris is little explored during NMES sessions, and the behavior of its components (RF, VL, VM and VI) is a lack to be studied by comparing the possible placement of the electrodes. The

purpose of this study was to investigate whether there is a difference in the behavior of the RF, VL, VM, and vastus intermedius (VI) during an evoked contraction with the electrodes placed over the motor point of the VL and VM. Once the muscle length was standardized for isometric contractions, we hypothesized that any difference in the degree of increase in θ_p, as well reduction in L_f, would be greater in the VL and VM constituent due to a direct electrode position during an evoked contraction. This knowledge may help physical therapists to establish a methodologic impact for future studies that might lead to a better understanding of evoked torque related to the positions of the electrodes for potential clinical NMES quadriceps activation.

2- MATERIAL AND METHODS

2.1- Participants

Twenty men with no known neuromuscular disorders and not engaged in systematic lower limb strengthening or sports competitions in the previous six months, physically active according to International Physical Activity Questionnaire (IPAQ) and with a minimum torque range of 40% of the maximum voluntary isometric contraction during NMES participated in the study. Sample size (n = 20) was determined a priori using G* POWER (v 3.13; University of Trier, Germany). The level of significance was set at $p = 0.05$, a power $(1- \beta) = 0.80$, and an effect size = 0.75. Subjects were informed about the purposes, benefits, and risks before enrollment, and all agreed to participate and signed the consent form. Approval was obtained (protocol number 94388718.8.0000.8093) from the Research Ethics Committee of the University of Brasília/Faculty of Ceilândia following the Helsinki Declaration of 1975.

2.2- Experimental design

This is a sub-study of a cross-over larger trial dealing with muscle-tendon behavior at hip and knee angles during maximum evoked and voluntary contractions. The full protocol can be assessed with the ClinicalTrials.gov (Identifier: NCT03822221). The volunteers were instructed not to participate in exhaustive activities 48 hours before the tests, sleep, and eat properly. The procedures took place in two visits, separated by a period of seven days. In the first visit, the volunteers were familiarized with the research procedures to reduce performance variability. In this visit, characterization variables were also obtained: height, weight, and the IPAQ, the passive measures of θ_p and L_f with ultrasound, as well as the motor point localization on the VL and VM, using a pen electrode, as described by Botter et al.[23] The location of each motor point was recorded as the distance from the patellar base and the thigh midline to be reproduced in the following sessions. Subsequently, two maximum voluntary contractions (MVC) and two maximum evoked isometric contractions (MEIC) were performed to verify if participants tolerated a current amplitude enough to generate an MEIC $\geq$ 40% of the MVC and to familiarize the participant with the dynamometer and verify responsiveness and comfort during NMES.

The second visit was the experimental session, with a minimum of seven days rest after the familiarization. Before the beginning of this session, a warm-up of 6 submaximal isometric contractions of 5 seconds and a 10-second rest interval between them (three to 50%, two to 75%, and one to 90% of the maximum effort perception) and another two maximal voluntary contractions with two minutes rest between them was conducted. All participants were submitted to eight MEIC at a rate of 1 per minute, which was the number of contractions required to assess all quadriceps constituents (two contractions for each one). The variables observed were θ_p and L_f at rest and evoked contraction at 40% of MVC.

2.3- Analysis at 40% of maximum voluntary contraction

To synchronize the torque tracing with the ultrasonographic recordings, we used a data acquisition device, New Miotool (Miotec Biomedical Equipment Ltd., POA, Brazil®) collected with a sampling rate of 2000 Hz per channel, A/D converter of 14 bits, common rejection mode of 110 dB (at 60 Hz). For this, the data acquisition device was connected to the computerized dynamometer, and a high-definition camera was positioned to capture the ultrasound display. When the assessor started recording cine-loop ultrasound images prior to contraction, a visual indicator appeared on the ultrasound screen, which enabled the synchronization of all data on a torque-time recording generated by the device.[24] In this torque-time screen, it was possible to move a cursor to the point where the torque was 40% of the MVC. In the time displayed, as well as in rest, we obtained a print image from the ultrasonographic video files using the Tracker 4.87 software for the calculation of θ_p and L_f using the ImageJ software (v. 1.46; National Institutes of Health, Bethesda, Maryland).

2.4- Torque

The Biodex System 4 ™ isokinetic dynamometer (Biodex Medical Systems, Inc, Shirley, New York) assessed the torque during voluntary contraction and NMES. The individuals were positioned on the isokinetic dynamometer chair with the hip flexed at 85º of flexion and the knee at 60º of flexion. The equipment axis was aligned with the anatomical axis of the knee and the lever arm with a force transducer, which was firmly fixed 2-3 cm above lateral malleolus with a strap. Each subject was firmly stabilized on the test chair with two belts crossing the chest and one crossing the pelvic girdle to minimize any unwanted body movement during strength production. The resting torque was used for subsequent gravity correction due to the limb weight and forces from the passive tension of structures crossing the knee.[25] The number of contractions

corresponded to the number of ultrasound assessments: four muscles (VL, VM, VI, and RF), with two measurements for each = eight evoked contractions, which were separated by one minute of rest.

2.5- NMES

The Neurodyn 2.0 electrical stimulation unit (Ibramed, SP, Brazil) was connected to two isolated cables. All physical parameters of the stimulator were verified using a digital oscilloscope (DS1050E, Rigol, Ohio, USA). The self-adhesive electrodes of 25 cm^2 were placed on the motor point of VL and VM. A pulsed current was applied, with a frequency of 100 Hz, pulse duration 400 μs, rise time of three seconds, ON time of four seconds, the decay time of three seconds, and OFF time of two minutes. The specifications of the ON time were designed to mimic a ramp contraction and allow the quadriceps unit assessment with ultrasound imaging as recommended in voluntary contractions.[26] To achieve the evoked contraction, subjects were instructed to fully relax during NMES. The current amplitude was gradually increased. Participants reported their discomfort after each evoked contraction using a 0–10 numeric scale, where 0 represented no discomfort and 10 represented the maximum discomfort they could support. According to a previous study, participants were informed that a report of 8 out of 10 of perceived discomfort should correspond to the maximum tolerated current amplitude they were willing to tolerate.[27] After achieving the maximum tolerated current amplitude, eight evoked contractions were obtained to allow all ultrasonographic recordings. In the primary study,[11] the range of electrical intensity tolerated by all volunteers corresponded to a torque generation at 40% of the MVC.

2.6- Ultrasonography

θ_p and L_f were obtained using a portable ultrasound device (M-Turbo®, Sonosite, Bothwell, WA, USA) in B mode with a 7.5 MHz linear transducer. Depth (6 cm, programmed in the device), compression, and stabilization of the transducer were kept constant between evaluations (by means of an apparatus made with styrofoam and velcro). Two videos were obtained for each quadriceps component. The transducer was positioned in the longitudinal plane of muscle, keeping it parallel with the direction of muscle fascicles. Proper transducer alignment was achieved when several fascicles were tracked without interruption. The lateral compartment of bipennate RF, VL, and VM were evaluated, respectively, in the percentages 50%, 60%, and 75% of the distance between the medial aspect of anterosuperior iliac spine and upper edge of patella, starting from proximal to distal, as adapted from Blazevich, Gill, and Zhou.[14] For the VI, although it could be seen on the same window of the RF or VL [14] VI visualization was often partially lost during contraction. Thus, it was recorded more distally in the anterior aspect of the thigh. The RF also was seen in the anterior aspect of the thigh. The VL was viewed by sliding the transducer in a lateral direction, five cm from the midline, and VM was considered with the transducer at three cm in the medial direction of the thigh. Video files recorded during the contractions evoked were transferred to a computer for processing in public domain software (ImageJ software v. 1.46; National Institutes of Health, Bethesda, Maryland). The θ_p was calculated considering the angle between deep aponeurosis and fascicles. The L_f was directly measured whenever possible, or in cases where the fascicles extended beyond the visible field of view, linear extrapolation was applied.[28] The figure 1 shows an example of measurement at rest and during evoked contraction of muscle architecture.

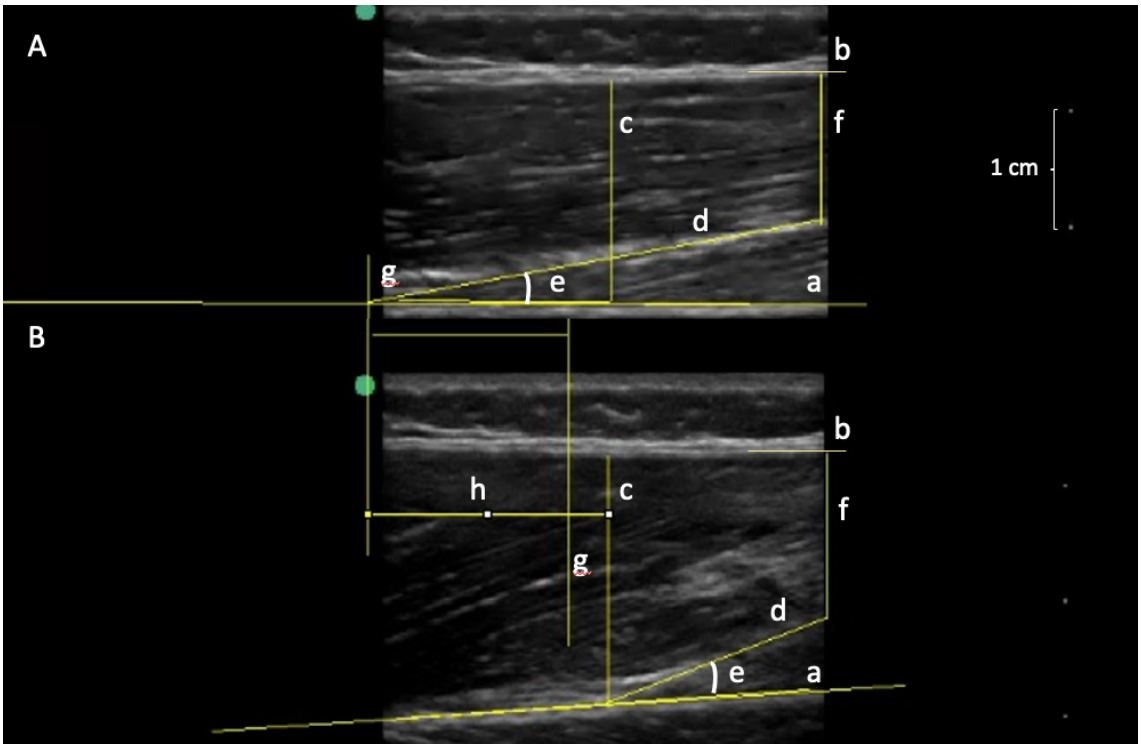

Figure 1 - Muscle ultrasonography: An ultrasound analysis of the vastus lateralis at rest (A) and during NMES-evoked contraction (B). (a) deep aponeurosis; (b) superficial aponeurosis; (c) muscle thickness; (d) fascicle with extrapolation or not; (e) pennation angle; (f) distance between end of fascicle visualization and superficial aponeurosis; (g) lines indicating the cross point between fascicle and deep aponeurosis; (h) tendon-aponeurosis complex displacement. Legend: NMES: neuromuscular electrical stimulation.

2.7- Statistical Analysis

Values of θ_p and L_f, are reported as mean ± standard deviation (SD). For θ_p and L_f, analyses were performed with the rest and contracting values (starting from rest up to 40% of the maximum voluntary torque). A two-way ANOVA was performed to verify the interaction between "muscle" (RF, VL, VM, and VI), and "contraction" (rest and evoked contraction at 40% of MVC) for the θ_p and L_f. And one-way ANOVA was fulfilled to verify the interaction between "muscle" during evoked contraction at 40% of MVC for the θ_p and L_f. When a significant difference was detected, the Tukey post-hoc test was applied to identify differences. The effect sizes and statistical power were

calculated. The effect size was determined using the partial square eta (ηρ2): small (ηρ2 = 0.01), medium (ηρ2 = 0.06) and large (ηρ2 = 0.14). The significance threshold was set at $P < 0.05$ for all procedures. All analyzes were performed using Statistica 23.0 (StatSoft Inc., Tulsa, Oklahoma, USA), and Graphpad Prism 8.3.0 software (San Diego, CA, USA) was used for graphic design.

3- RESULTS

Twenty men (mean ± SD age: 24.0 ± 4.6 years, body mass: 77.0 ± 9.3 kg, height: 177.6 ± 6.3 cm) participated of the study. The mean torque observed was 201.14 ± 50.22 N.m during MVC, and torque at 40% of MVC was 80.45 ± 20.08 N.m, as shown in table 1.

Table 1. MVC torque 40% and 100%

Participants	40% Torque MVC	100% Torque MVC
1	111,19	277,98
2	76,40	191
3	89,78	224,45
4	62,99	157,48
5	104,21	260,53
6	66,93	167,34
7	72,46	181,15
8	78,02	195,07
9	75,06	187,65
10	99,45	248,64
11	89,50	223,76
12	64,96	162,40
13	60,56	151,40
14	117,78	294,45
15	67,25	168,13
16	49,95	124,89
17	64,01	160,04
18	75,52	188,80
19	117,52	293,82
20	65,53	163,83
Mean	80,45	201,14
SD	20,08	50,22

Values expressed in absolute form. Legend: MVC: maximum voluntary contraction.

Figure 2 shows the mean and SD of θ_p and L_f.

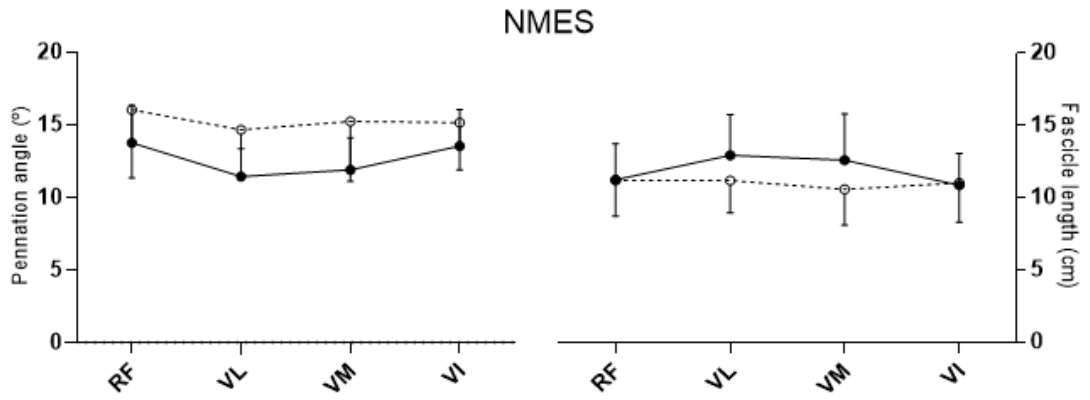

Figure 2 - Changes in pennation angle and fascicle length of components of quadriceps femoris during neuromuscular electrical stimulation at 40% of the maximum voluntary torque: fascicle length (right y-axis) and pennation angle (left y-axis) of all constituents of the quadriceps femoris individually (x-axis) at rest (continuous line) and during NMES (dotted line). Data are presented as mean ± SD. Legend: NMES: neuromuscular electrical stimulation; RF: rectus femoris; VL: vastus lateralis; VM: vastus medialis; VI: vastus intermedius.

Table 2 shows the changes in θ_p and L_f of four quadriceps femoris' components (RF, VL, VM, and VI) during evoked contraction.

Table 2. Fascicle length and pennation angle of the rectus femoris, vastus lateralis, vastus medialis and vastus intermedius at rest and during neuromuscular electrical stimulation at 40% of maximum voluntary contraction.

	Rest		NMES
Rectus femoris			
θ_p	13.74 ± 2.57		16.00 ± 4.68
		(17.36 ± 30.76%)	
L_f	11.25 ± 2.49		11.21 ± 2.47
		(2.54 ± 26.28%)	
Vastus lateralis			
θ_p	11.42 ± 1.91		14.64 ± 3.26
		(31.11 ± 32.92%)	
L_f	12.95 ± 2.81		11.20± 2.23
		(-11.09 ± 20.69%)	
Vastus medialis			
θ_p	11.87 ± 2.21		15.20± 4.10
		(35.69 ± 42.09%)	
L_f	12.61 ± 3.18		10.58± 2.46
		(-12.87 ± 24.76%)	
Vastus intermedius			
θ_p	13.52 ± 2.49		15.12 ± 3.25
		(13.35 ± 23.52%)	
L_f	10.87 ± 2.20		11.03± 2.68
		(3.23 ± 25.57%)	

Values expressed as mean ± SD. Legend: θ_p: pennation angle; L_f: fascicle length; NMES: neuromuscular electrical stimulation.

There was no interaction for muscle x contraction for θ_p ($F3.57 = 1.33$, $p = 0.27$, $\eta\rho2$: 0.06, power: 0.33). However, in a secondary analysis, we observed at ANOVA one-way a significant interaction for contraction showing an increase of θ_p ($F1.19 = 26.42$, $p < 0,05$, $\eta\rho2$: 0.58, power: 0.99) for each quadriceps components. Analyzing L_f ANOVA

two-way showed interaction for muscle x contraction (F3. 57 = 3.17, $p < 0.05$, $\eta\rho 2$: 0.14, power: 0.70). The Tukey post-hoc indicated only interactions not relevant to the study.

4- DISCUSSION

The objective of this study was to investigate whether during an NMES session the placement of the electrodes over the motor point of the VL and VM produce a different alteration in the muscle architecture in the behavior of quadriceps components, because it is the major positioning used in literature, although there is another possible configuration with the electrodes positioned over the RF.

NMES capacity to increase muscle strength is already a consensus in the literature. Kern et al.[29] found that after a two-year training with NMES in the lower limb of individuals with a complete lesion of the lower motor neuron, the quadriceps femoris had a 35% increase in its cross-sectional area, in addition to an increase of 1187% in the production of strength, which allowed patients to perform standing exercises assisted by electrostimulation. And one of the main factors that influence the force generation capacity is the muscle architecture,[8-10] because it provides biomechanical characteristics that are specific to a muscle and determines its function.

Muscle architecture has been defined as “the organization of muscle fibers within a muscle concerning the axis of force generation”. It is also one of the main manners of measuring muscle function.[9,30] One device commonly used to predict the capacity of a muscle to generate force is surface electromyography by motor unit activation.[31] However, the artifacts produced by the external electrical current are a common limitation to the interpretation of the electromyographic analysis. Thus, ultrasonography seems a feasible tool to obtain measures of muscle force production, as previous studies have postulated that, for isometric contractions of the same intensity for

a given joint angle, differences in the amount of changes on θp or Lf reflect differences in the contribution of that muscle for the observed torque.[30,32]

Blazevich et al.,[14] used ultrasonography to observe quadriceps femoris muscle architecture in vivo, but did not evaluate at contraction, only at rest. As, the study by Carbonaro et al.[33] related ultrasound and NMES to assess the architecture of the superficial and deep compartment of the anterior tibialis, suggesting that electrical stimulation through the muscle belly using surface electrodes recruits more superficial motor units. Another study correlated these two tools,[34] but ultrasound was used only at rest as a way to assess hypertrophy gains after training with NMES of four and eight weeks. Therefore, there is a lack of studies evaluating muscle architecture variables during an NMES session of quadriceps femoris.

The amplitude of the current is the only way to increase the generation of torque produced by the evoked contraction, from the moment the frequency and pulse width are defined.[35] However, the current amplitude is directly related to the discomfort perceived by the subjects. When the current intensity is increased, both the motor fibers and the nociceptive sensory fibers are stimulated, which can lead to a sensation of pain and burning. Therefore, if an increase in the generation of evoked torque is desired, the therapist can be limited to increasing, because along with this, the discomfort also increases.[18] Due to this, using efficient currents is a way to overcome the perceived discomfort.

Previous studies have already reported that an NMES training intensity of 40% MVC is capable of improving MVC strength.[3,36] Besides Pinto et al.[37] demonstrated when the discomfort perceived is reported at as 6 in the Visual Analog Scale, the torque evoked corresponds to 40% of MVC. Therefore, a NMES session using a moderate current amplitude is an efficient way to introduce this resource in a rehabilitation protocol.

Our main finding was no difference in muscle architecture of the components of quadriceps femoris promoted by electrostimulation at 40% of MVC with the electrodes positioned over the motor point of VL and VM, which is clinically important once it was already reported in the literature that an NMES training intensity of 40% MVC is capable of improving MVC strength.[3,36] It indicates that independently of the electrodes placement, NMES can produce significant gains on quadriceps femoris strength.

Some limitations of the study should be mentioned: the exact observation of L_f's start and end point during evoked contraction may have varied because NMES promoted a sudden muscle contraction, and the transducer was held by the examiner, making it challenging to acquire an accurate image. In addition, our ultrasound had a probe width of 40 mm, which limited the visualization of all muscle fascicles. Finally, our results are limited to our population and a single NMES session.

5- CONCLUSION

There is no difference in the behavior of the components of the quadriceps femoris during stimulation with the electrodes placed on the motor points of the VL and VM, even as there were no acute changes in the quadriceps muscle architecture, which suggests that NMES can be used with this configuration of electrodes because all the quadriceps muscles undergo similar acute changes. Besides, more studies are necessary to analyze chronic changes in architecture in long-term training to confirm the findings of this study.

7- REFERENCES

1- Taradaj J, Halski T, Kucharzewski M, Walewicz K, Smykla A, Ozon M, et al. The effect of neuromuscular electrical stimulation on quadriceps strength and knee function in professional soccer players: return to sport after ACL reconstruction. *Biomed Res Int*. 2013;2013:802534. doi:10.1155/2013/802534.

2- Toth MJ, Tourville TW, Voigt TB, Choquette RH, Anair BM, Falcone MJ, et al. Utility of Neuromuscular Electrical Stimulation to Preserve Quadriceps Muscle Fiber Size and Contractility After Anterior Cruciate Ligament Injuries and Reconstruction: A Randomized, Sham-Controlled, Blinded Trial. *Am J Sports Med*. 2020;48(10):2429-37. doi:10.1177/0363546520933622.

3- Maffiuletti NA, Minetto MA, Farina D, Bottinelli R. Electrical stimulation for neuromuscular testing and training: state-of-the art and unresolved issues. *Eur J Appl Physiol*. 2011;111(10):2391-7. doi:10.1007/s00421-011-2133-7.

4- Maffiuletti NA. Physiological and methodological considerations for the use of neuromuscular electrical stimulation. *Eur J Appl Physiol*. 2010;110(2):223-34. doi:10.1007/s00421-010-1502-y.

5- Barss TS, Ainsley EN, Claveria-Gonzalez FC, Luu MJ, Miller DJ, Wiest MJ, et al. Utilizing Physiological Principles of Motor Unit Recruitment to Reduce Fatigability of Electrically-Evoked Contractions: A Narrative Review. *Arch Phys Med Rehabil*. 2018;99(4):779-91. doi:10.1016/j.apmr.2017.08.478.

6- Bastos JAI, Martins W, Junior GC, Collins DF, Durigan JLQ. Contraction fatigue, strength adaptations, and discomfort during conventional versus wide-pulse, high-frequency, neuromuscular electrical stimulation: a systematic review. *Appl Physiol Nutr Metab*. 2021. doi:10.1139/apnm-2021-0269.

7- Simoneau-Buessinger E, Leteneur S, Bisman A, Gabrielli F, Jakobi J. Ultrasonographic quantification of architectural response in tibialis anterior to neuromuscular electrical stimulation. *J Electromyogr Kinesiol*. 2017;36:90-5. doi:10.1016/j.jelekin.2017.07.008.

8- Fukunaga T, Kawakami Y, Kuno S, Funato K, Fukashiro S. Muscle architecture and function in humans. *J Biomech*. 1997;30(5):457-63. doi:10.1016/s0021-9290(96)00171-6.

9- Lieber RL, Bodine-Fowler SC. Skeletal muscle mechanics: implications for rehabilitation. *Phys Ther*. 1993;73(12):844-56. doi:10.1093/ptj/73.12.844.

10- Seymore KD, Domire ZJ, DeVita P, Rider PM, Kulas AS. The effect of Nordic hamstring strength training on muscle architecture, stiffness, and strength. *Eur J Appl Physiol*. 2017;117(5):943-53. doi:10.1007/s00421-017-3583-3.

11- Cavalcante JGT, Marqueti RC, Geremia JM, de Sousa Neto IV, Baroni BM, Silbernagel KG, et al. The Effect of Quadriceps Muscle Length on Maximum Neuromuscular Electrical Stimulation Evoked Contraction, Muscle Architecture, and Tendon-Aponeurosis Stiffness. *Front Physiol*. 2021;12:633589. doi:10.3389/fphys.2021.633589.

12- Reeves ND, Narici MV. Behavior of human muscle fascicles during shortening and lengthening contractions in vivo. *J Appl Physiol* (1985). 2003;95(3):1090-6. doi:10.1152/japplphysiol.01046.2002.

13- Ando R, Nosaka K, Inami T, Tomita A, Watanabe K, Blazevich AJ, et al. Difference in fascicle behaviors between superficial and deep quadriceps muscles during isometric contractions. *Muscle Nerve*. 2016;53(5):797-802. doi:10.1002/mus.24905.

14- Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat*. 2006;209(3):289-310. doi:10.1111/j.1469-7580.2006.00619.x.

15- Bergquist AJ, Wiest MJ, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: quadriceps femoris. *J Appl Physiol* (1985). 2012;113(1):78-89. doi:10.1152/japplphysiol.00074.2011.

16- Lepley LK, Wojtys EM, Palmieri-Smith RM. Combination of eccentric exercise and neuromuscular electrical stimulation to improve quadriceps function post-ACL reconstruction. *Knee*. 2015;22(3):270-7. doi:10.1016/j.knee.2014.11.013.

17- Labanca L, Rocchi JE, Laudani L, Guitaldi R, Virgulti A, Mariani PP, et al. Neuromuscular Electrical Stimulation Superimposed on Movement Early after ACL Surgery. *Med Sci Sports Exerc*. 2018;50(3):407-16. doi:10.1249/MSS.0000000000001462.

18- Glaviano NR, Saliba S. Can the Use of Neuromuscular Electrical Stimulation Be Improved to Optimize Quadriceps Strengthening? *Sports Health*. 2016;8(1):79-85. doi:10.1177/1941738115618174.

19- Kim KM, Croy T, Hertel J, Saliba S. Effects of neuromuscular electrical stimulation after anterior cruciate ligament reconstruction on quadriceps strength, function, and patient-oriented outcomes: a systematic review. *J Orthop Sports Phys Ther*. 2010;40(7):383-91. doi:10.2519/jospt.2010.3184.

20- Vieira TM, Potenza P, Gastaldi L, Botter A. Electrode position markedly affects knee torque in tetanic, stimulated contractions. *Eur J Appl Physiol*. 2016;116(2):335-42. doi:10.1007/s00421-015-3289-3.

21- Pietrosimone BG, Selkow NM, Ingersoll CD, Hart JM, Saliba SA. Electrode type and placement configuration for quadriceps activation evaluation. *J Athl Train*. 2011;46(6):621-8. doi:10.4085/1062-6050-46.6.621.

22- Torry MR, Decker MJ, Viola RW, O'Connor DD, Steadman JR. Intra-articular knee joint effusion induces quadriceps avoidance gait patterns. *Clin Biomech* (Bristol, Avon). 2000;15(3):147-59. doi:10.1016/s0268-0033(99)00083-2.

23- Botter A, Oprandi G, Lanfranco F, Allasia S, Maffiuletti NA, Minetto MA. Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning. *Eur J Appl Physiol*. 2011;111(10):2461-71. doi:10.1007/s00421-011-2093-y.

24- Bojsen-Møller J, Hansen P, Aagaard P, Kjaer M, Magnusson SP. Measuring mechanical properties of the vastus lateralis tendon-aponeurosis complex in vivo by ultrasound imaging. *Scand J Med Sci Sports*. 2003;13(4):259-65. doi:10.1034/j.1600-0838.2003.00301.x.

25- Bampouras TM, Reeves ND, Baltzopoulos V, Maganaris CN. The role of agonist and antagonist muscles in explaining isometric knee extension torque variation with hip joint angle. *Eur J Appl Physiol*. 2017;117(10):2039-45. doi:10.1007/s00421-017-3693-y.

26- Stafilidis S, Tilp M. Effects of short duration static stretching on jump performance, maximum voluntary contraction, and various mechanical and morphological parameters of the muscle-tendon unit of the lower extremities. *Eur J Appl Physiol*. 2015;115(3):607-17. doi:10.1007/s00421-014-3047-y.

27- Scott W, Adams C, Fisher J, Fisher S, Jones K, Mathieu B. Electrically elicited quadriceps muscle torque: Comparison at three knee angles. *Physiother Theory Pract*. 2021;37(6):729-35. doi:10.1080/09593985.2019.1639867.

28- Narici MV, Binzoni T, Hiltbrand E, Fasel J, Terrier F, Cerretelli P. In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *J Physiol*. 1996;496 (Pt 1):287-97. doi:10.1113/jphysiol.1996.sp021685.

29- Kern H, Carraro U, Adami N, Biral D, Hofer C, Forstner C, et al. Home-based functional electrical stimulation rescues permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. *Neurorehabil Neural Repair*. 2010;24(8):709-21. doi:10.1177/1545968310366129.

30- Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve*. 2000;23(11):1647-66. doi:10.1002/1097-4598(200011)23:11<1647::aid-mus1>3.0.co;2-m.

31- Chowdhury RH, Reaz MB, Ali MA, Bakar AA, Chellappan K, Chang TG. Surface electromyography signal processing and classification techniques. *Sensors* (Basel). 2013;13(9):12431-66. doi:10.3390/s130912431.

32- Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Magnusson SP, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol*. 2001;534(Pt. 2):613-23. doi:10.1111/j.1469-7793.2001.t01-1-00613.x.

33- Carbonaro M, Seynnes O, Maffiuletti NA, Busso C, Minetto MA, Botter A. Architectural Changes in Superficial and Deep Compartments of the Tibialis Anterior During Electrical Stimulation Over Different Sites. *IEEE Trans Neural Syst Rehabil Eng*. 2020;28(11):2557-65. doi:10.1109/TNSRE.2020.3027037.

34- Gondin J, Guette M, Ballay Y, Martin A. Electromyostimulation training effects on neural drive and muscle architecture. *Med Sci Sports Exerc*. 2005;37(8):1291-9. doi:10.1249/01.mss.0000175090.49048.41.

35- Low J, Reid A. Electrotherapy Explained. Butterworth-Heinemann, London. 1990.

36- Lieber RL, Kelly MJ. Factors influencing quadriceps femoris muscle torque using transcutaneous neuromuscular electrical stimulation. *Phys Ther*. 1991;71(10):715-21; discussion 22-3. doi:10.1093/ptj/71.10.715.

37- Pinto Damo NL, Modesto KA, Neto IVS, Bottaro M, Babault N, Durigan JLQ. Effects of different electrical stimulation currents and phase durations on submaximal and maximum torque, efficiency, and discomfort: a randomized crossover trial. *Braz J Phys Ther*. 2021. doi:10.1016/j.bjpt.2021.03.001.

8- APPENDICES

8.1- Research ethics committee approval

UNB - FACULDADE DE CEILÂNDIA DA UNIVERSIDADE DE BRASÍLIA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos agudos da estimulação elétrica neuromuscular em diferentes ângulos do quadril e do joelho nas adaptações neuromiotendíneas e no torque extensor do joelho em adultos jovens saudáveis

Pesquisador: Jonathan Galvão Tenório Cavalcante

Área Temática:

Versão: 1

CAAE: 94388718.8.0000.8093

Instituição Proponente: Universidade de Brasília Faculdade de Ceilândia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.799.049

Apresentação do Projeto:

Trata-se de um projeto mestrado do Programa de Pós Graduação em Ciências da Reabilitação, da Faculdade de Ceilândia – Universidade de Brasília, do mestrando Jonathan Galvão Tenório Cavalcante, e demais pesquisadores envolvidos: Karenina Arrais Guida Modesto, Rita de Cássia Marqueti Durigan, Nicolas Babault e João Luiz Quagliotti Durigan (orientador). Tem como Área de Estudo (Grandes Áreas do Conhecimento (CNPq): Grande Área 4. Ciências da Saúde e o Propósito Principal do Estudo (OMS): Clínico. O pesquisador deixa claro quais são os problemas ou Condições de saúde (Plasticidade musculotendínea, Força muscular, Relação comprimento-tensão, Atividade elétrica muscular) vinculando ao CID: M62.9 - Transtorno muscular não especificado e, M62 - Outros transtornos musculares.

Os pesquisadores informam que:

"Trata-se de um estudo observacional de delineamento transversal. As variáveis independentes são: 1) o posicionamento do membro inferior: angulação da articulação do joelho em 20° ou 60° com o quadril em 0° ou 80° (respectivamente, indivíduos deitados ou sentados com leve reclinação); 2) a estimulação elétrica neuromuscular para obtenção do torque evocado. As variáveis dependentes serão a contração voluntária máxima (CVM), o torque evocado eletricamente (TEE), a atividade elétrica de superfície, a arquitetura muscular (espessura muscular, ângulo de penação e comprimento fascicular) e o alongamento do complexo tendão-aponeurose do quadríceps e a

Endereço: UNB - Prédio da Unidade de Ensino e Docência (UED), Centro Metropolitano, conj. A, lote 01, Sala AT07/66
Bairro: CEILANDIA SUL (CEILANDIA) **CEP:** 72.220-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-8434 **E-mail:** cep.fce@gmail.com

rigidez, módulo de Young e área de secção transversa do tendão patelar. Os torques gerados na CVM e no TEE serão comparados entre os diferentes ângulos do joelho, enquanto que as demais variáveis serão comparadas em duas situações: em repouso entre os diferentes ângulos do joelho e em repouso versus em contração."

Ainda descrevem no projeto que "A estimulação elétrica neuromuscular (NMES) é um recurso não-invasivo que ativa o músculo esquelético artificialmente e, dessa forma, perpassa o trajeto natural da contração muscular (Trimble & Enoka, 1991). A NMES é amplamente usada na reabilitação para auxiliar na recuperação de força, reeducação muscular, redução de atrofia muscular e redução de limitações funcionais (Salvini et al., 2012; Vaz et al., 2013, Durigan et al., 2014a,b; Oliveira et al., 2018a; Almeida et al., 2018), bem como também é usada entre indivíduos saudáveis e atletas para o aumento de força muscular e desempenho esportivo (Bax et al., 2005; Billot et al., 2010)."

Objetivo da Pesquisa:

Objetivo Primário:

• Investigar o efeito da estimulação elétrica neuromuscular – NMES, em diferentes ângulos do quadril e do joelho no torque extensor, na atividade eletromiográfica, na arquitetura muscular, no complexo tendão-aponeurose e nas propriedades tendíneas dos componentes do quadríceps.

Objetivos específicos

Analisar o torque de extensão do joelho durante a estimulação elétrica neuromuscular tendo como intervenção controle a contração voluntária isométrica máxima;

Analisar a atividade elétrica dos três componentes superficiais do quadríceps (vasto lateral, reto femoral e vasto medial) em repouso e durante contração máxima, bem como do antagonista bíceps femoral;

Analisar a arquitetura muscular (ângulo de penação, comprimento fascicular e espessura muscular) dos quatro componentes do quadríceps femoral em repouso e durante contração máxima;

Analisar a rigidez do complexo tendão-aponeurose do tendão quadricipital em repouso e durante as manobras de contração máxima;

Testar as variáveis acima em quatro posturas diferentes: joelho em 0°, 60° e 90°, estando o quadril em 80°, e ambos o joelho e o quadril em 0° (ou seja, em extensão, estando o indivíduo em decúbito dorsal).

Avaliação dos Riscos e Benefícios:

RISCOS

• Reações alérgicas e/ou queimadura a nível tecidual nas regiões selecionadas para avaliação da excitabilidade neuromuscular inerente à estimulação elétrica;

• Lesão neuromuscular ou tendínea decorrente da estimulação elétrica;

• Constrangimento durante as avaliações;

• Fadiga muscular.

BENEFÍCIOS:

• Os participantes receberão, de forma impressa ou por email, os resultados dos exames de imagem e eletromiográficos realizados e serão explicados o significado e as repercussões conforme a literatura atual;

• Os indivíduos terão a força dos extensores do joelho avaliada por meio de um dinamômetro isocinético e saberão se se encontram na faixa esperada para a sua faixa etária e altura;

• No caso de que alterações musculoesqueléticas sejam observadas, mesmo que estas impossibilitem o indivíduo de participar do estudo, será realizada avaliação fisioterapêutica mais aprofundada e orientações serão fornecidas quanto ao tratamento e a necessidade de consulta médica.

• Após finalização do estudo e publicação, cada participante receberá a versão em PDF do artigo, bem como terão livre acesso à dissertação no acervo da Biblioteca da Universidade de Brasília.

Medidas de proteção de risco e outros aspectos éticos

• Serão utilizados materiais de uso comum em pesquisas e na prática clínica, como gel condutor, gaze e eletrodo de carbono, os quais não possuem histórico de proporcionar reações adversas ao contato com a epiderme íntegra;

• O risco de lesão pela corrente da estimulação elétrica será minimizado por meio do incremento controlado da intensidade do estímulo e pela observação da reação muscular e da presença de hiperemia na pele;

• Será respeitada a autonomia do participante durante todo o processo, podendo o mesmo se recusar a qualquer momento, caso não se sinta apto a realizar os procedimentos. Os resultados serão transmitidos de forma apropriada, adaptada à condição física, emocional e intelectual do indivíduo;

• Será garantido o sigilo profissional e todos os participantes serão identificados por números, sendo ocultados seus nomes dos formulários de avaliação;

• As contrações musculares serão separadas por um período suficiente de repouso para evitar fadiga muscular.

Comentários e Considerações sobre a Pesquisa:

"O tamanho da amostra foi determinado do por meio do software G* Power (versão 3.13; Universidade de Trier, Alemanha): 20 sujeitos serão recrutados, considerando o pico de torque extensor voluntário 60° de flexão do joelho como a variável principal, de acordo com estudo previamente publicado (Ruiter et al., 2008), o qual mostra uma média de 107.3 Nm ± 20.7. Para isso, o nível de significância foi estabelecido em 5% (p= 0,05), um power (1-) = 0,80 e um tamanho de efeito = 0,90. Os indivíduos serão recrutados pelo método de amostragem não probabilística de conveniência, por meio de panfletos a serem distribuídos na Universidade e por meio de convite verbal."

Os pesquisadores deixam claro os critérios de inclusão e exclusão:

Critério de Inclusão:

Serão incluídos indivíduos idade entre 18 e 30 anos, do sexo masculino, hígidos e com índice de massa corpórea (IMC) 18,5 - 24,9 kg/m²; não participantes de treino sistemático de fortalecimento dos membros inferiores nos últimos 12 meses; praticantes ou não de atividades esportivas recreativamente; fisicamente ativos de acordo com o Questionário Internacional de Atividade Física (IPAQ); e com alcance de torque mínimo de 30% da contração voluntária isométrica máxima durante a NMES.

Critério de Exclusão:

Serão excluídos aqueles que apresentarem: dor em qualquer dos procedimentos; edema, lesão dérmica, limitação da amplitude de movimento articular, deformidade ou amputação em qualquer parte dos membros inferiores; histórico de luxação patelar ou trauma nos membros inferiores ou tronco que comprometa os resultados; condições que afetem a morfologia musculotendínea ou a excitabilidade neuromuscular como diabetes mellitus tipo II, hipercolesterolemia familiar, doença neuromuscular e cardiopatia grave; déficit cognitivo, doença psiquiátrica, dependência química ou problemas comportamentais que inviabilizem a cooperação com os procedimentos (Dudley-Javoroski et al., 2010).

A pesquisa terá um custo de R$280.503,75, e o pesquisador deixa claro que esse custo refere-se, na maior parte aos equipamentos cedidos pela Faculdade da Ceilândia (FCE) da Universidade de

Brasília, e os menores gastos serão arcados pelos pesquisadores.

Considerações sobre os Termos de apresentação obrigatória:

Todos os documentos foram adequadamente apresentados.

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências.

Considerações Finais a critério do CEP:

Protocolo de pesquisa em consonância com a Resolução 466/12 do Conselho Nacional de Saúde. Cabe ressaltar que compete ao pesquisador responsável: desenvolver o projeto conforme delineado; elaborar e apresentar os relatórios parciais e final; apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento; manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa; encaminhar os resultados da pesquisa para publicação, com os devidos créditos aos 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_1109531.pdf	23/07/2018 13:21:53		Aceito
Outros	Curriculo_Nicolas_Babault_ingles.pdf	23/07/2018 13:21:18	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	Carta_resposta_membro_estrangeiro.pdf	23/07/2018 13:19:48	Jonathan Galvão Tenório Cavalcante	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Detalhado.docx	16/07/2018 18:24:30	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	Curriculo_Lattes_Karenina_Arrais.pdf	16/07/2018 18:22:28	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	Curriculo_Lattes_Rita_de_Cassia.pdf	16/07/2018 18:21:41	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	Curriculo_Lattes_Joao_Luiz.pdf	16/07/2018 18:21:17	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	Curriculo_Lattes_Jonathan_Galvao.pdf	16/07/2018 18:20:36	Jonathan Galvão Tenório Cavalcante	Aceito

Endereço: UNB - Prédio da Unidade de Ensino e Docência (UED), Centro Metropolitano, conj. A, lote 01, Sala AT07/66
Bairro: CEILANDIA SUL (CEILANDIA) **CEP:** 72.220-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-8434 **E-mail:** cep.fce@gmail.com

Outros	termo_de_concordancia_de_instituicao_participante.pdf	16/07/2018 18:19:25	Jonathan Galvão Tenório Cavalcante	Aceito
Declaração de Pesquisadores	termo_de_responsabilidade_e_compromisso_do_pesquisador.pdf	16/07/2018 18:18:23	Jonathan Galvão Tenório Cavalcante	Aceito
Folha de Rosto	folhaDeRosto.pdf	16/07/2018 18:16:25	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	cartaencaminhprojeto_ao_cepfce.docx	28/06/2018 11:08:53	Jonathan Galvão Tenório Cavalcante	Aceito
Orçamento	Planilha_de_orcamento.doc	28/06/2018 02:24:55	Jonathan Galvão Tenório Cavalcante	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Jonathan_Galvao.doc	28/06/2018 02:22:51	Jonathan Galvão Tenório Cavalcante	Aceito
Cronograma	Cronograma.docx	28/06/2018 02:21:44	Jonathan Galvão Tenório Cavalcante	Aceito
Outros	termo_instituicao_proponente.jpg	28/06/2018 02:19:23	Jonathan Galvão Tenório Cavalcante	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRASILIA, 03 de Agosto de 2018

Assinado por:
Dayani Galato
(Coordenador)

8.2- Author Guidelines for submission

GUIDE FOR AUTHORS

INTRODUCTION

Types of article

The **Brazilian Journal of Physical Therapy (BJPT)** publishes original research articles, reviews, and brief communications on topics related to physical therapy and rehabilitation, including clinical, basic or applied studies on the assessment, prevention and treatment of movement disorders. Our Editorial Board is committed to disseminate high-quality research in the field of physical therapy. The BJPT follows the principle of publication ethics included in the code of conduct of the Committee on Publication Ethics (COPE).The BJPT accepts the submission of manuscripts with up to 3,500 words (excluding title page, abstract, references, tables, figures and legends). Information contained in appendices will be included in the total number of words allowed. A total of five (5) combined tables and figures is allowed.

The following types of study can be considered for publication, if directly related to the journals scope:
a) Intervention studies (clinical trials): studies that investigate the effect(s) of one or more interventions on outcomes directly related to the BJPTs scope. The World Health Organization defines a clinical trial as any research study that prospectively allocates human participants or groups of humans to one or more health-related interventions to evaluate the effect(s) on health outcome(s). Clinical trials include single-case experimental studies, case series, non-randomized controlled trials, and randomized controlled trials. Randomized controlled trials (RCTs) must follow the CONSORT (Consolidated Standards of Reporting Trials) recommendations, which are available at: http://www.consort-statement.org/consort-statement/overview0/. The CONSORT checklist and Statement Flow Diagram, available at http://www.consort-statement.org/consort-statement/flow-diagram, must be completed and submitted with the manuscript. Clinical trials must provide registration that satisfies the requirements of the International Committee of Medical Journal Editors (ICMJE), e.g. http://clinicaltrials.gov/ and/or http://www.anzctr.org.au. The complete list of all clinical trial registries can be found at: http://www.who.int/ictrp/network/primary/en/index.html. We suggest that all authors register clinical trials prospectively via the website http://www.clinicaltrials.gov.
Note: We do not accept single case studies and series of cases (i.e. clinical trials without a comparison group).

b) Observational studies: studies that investigate the relationship(s) between variables of interest related to the BJPTs scope. Observational studies include cross-sectional studies, cohort studies, and case-control studies. All observational studies must be reported following the recommendation from the STROBE statement (http://strobe-statement.org/index.php?id=strobe-home).

c) Qualitative studies: studies that focus on understanding needs, motivations, and human behavior. The object of a qualitative study is guided by in-depth analysis of a topic, including opinions, attitudes, motivations, and behavioral patterns without quantification. Qualitative studies include documentary and ethnographic analysis.

d) Systematic reviews: studies that analyze and/or synthesize the literature on a topic related to the scope of the BJPT. Systematic reviews that include meta-analysis will have priority over other systematic reviews. Those that have an insufficient number of articles or articles with low quality in the Methods section and do not include an assertive and valid conclusion about the topic will not be considered for peer-review analysis.
The authors must follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist to format their systematic reviews. The checklist is available at http://www.prisma-statement.org/PRISMAStatement/Default.aspx and must be filled in and submitted with the manuscript.
Potential authors are encouraged to read the following tutorial, which contains the minimum requirements for publication of systematic reviews in the BJPT: Mancini MC, Cardoso JR, Sampaio RF, Costa LCM, Cabral CMN, Costa LOP. Tutorial for writing systematic reviews for the Brazilian Journal of Physical Therapy (BJPT). Braz J Phys Ther. 2014 Nov-Dec; 18(6):471-480.

e) Studies on the translation and cross-cultural adaptation of questionnaires or assessment tools: studies that aim to translate and/or cross-culturally adapt foreign questionnaires to a language other than that of the original version of existing assessment instruments. The authors must use the

checklist (Appendix) to format this type of paper and adhere to the other recommendations of the BJPT. The answers to the checklist must be submitted with the manuscript. At the time of submission, the authors must also include written permission from the authors of the original instrument that was translated and/or cross-culturally adapted.

f) Methodological studies: studies centered on the development and/or evaluation of clinimetric properties and characteristics of assessment instruments. The authors are encouraged to use the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) to format methodological papers, in addition to following BJPT instructions. Important: Studies that report electromyographic results must follow the Standards for Reporting EMG Data recommended by ISEK (International Society of Electrophysiology and Kinesiology), available at http://www.isek.org/wp-content/uploads/2015/05/Standards-for-Reporting-EMG-Data.pdf.

g) Clinical trial protocols: The BJPT welcomes the publication of clinical trial protocols. We only accept trial protocols that are substantially funded, have ethics approval, have been prospectively registered and of very high quality. We expect that clinical trial protocols must be novel and with a large sample size. Finally, authors have to provide that the clinical trial is on its first stages of recruitment. Authors should use the SPIRIT statement while formatting the manuscript (http://www.spirit-statement.org). Funding solely based upon scholarships or fellowships are not considered as substantially funded.

h) Short communications: the BJPT will publish one short communication per issue (up to six a year) in a format similar to that of the original articles, containing 1200 words and up to two figures, one table, and ten references.

i) Masterclass articles: This type of article presents the state of art of any topic that is important to the field of physical therapy. All masterclass articles are invited manuscripts and the authors must be recognized experts in the field. However, authors can send e-mails to the editor in chief with an expression of interest to submit a masterclass article to the BJPT.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Clinical trial results

In line with the position of the International Committee of Medical Journal Editors, the journal will not consider results posted in the same clinical trials registry in which primary registration resides to be prior publication if the results posted are presented in the form of a brief structured (less than 500 words) abstract or table. However, divulging results in other circumstances (e.g., investors' meetings) is discouraged and may jeopardise consideration of the manuscript. Authors should fully disclose all posting in registries of results of the same or closely related work.

Reporting clinical trials

Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the randomization procedure. The CONSORT checklist and template flow diagram are available online.

Registration of clinical trials

Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with International Committee of Medical Journal Editors recommendations. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this) to assign to theAssociação Brasileira de Pesquisa e Pós-Graduação em Fisioterapia (ABRAPG-FT) the copyright in the manuscript and any tables, illustrations or other material submitted for publication as part of the manuscript (the "Article") in all forms and media (whether now known or later developed), throughout the world, in all languages, for the full term of copyright, effective when the Article is accepted for publication. An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher and ABRAPG-FT is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in this journal.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Open access
Please visit our Open Access page from the Journal Homepage for more information.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Informed consent and patient details
Studies on patients or volunteers require ethics committee approval and informed consent, which should be documented in the paper. Appropriate consents, permissions and releases must be obtained where an author wishes to include case details or other personal information or images of patients and any other individuals in an Elsevier publication. Written consents must be retained by the author but copies should not be provided to the journal. Only if specifically requested by the journal in exceptional circumstances (for example if a legal issue arises) the author must provide copies of the consents or evidence that such consents have been obtained. For more information, please review the Elsevier Policy on the Use of Images or Personal Information of Patients or other Individuals. Unless you have written permission from the patient (or, where applicable, the next of kin), the personal details of any patient included in any part of the article and in any supplementary materials (including all illustrations and videos) must be removed before submission.

Submission
Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article
Please submit your article via https://www.evise.com/profile/api/navigate/BJPT.

PREPARATION

Double-blind review
This journal uses double-blind review, which means the identities of the authors are concealed from the reviewers, and vice versa. More information is available on our website. To facilitate this, please include the following separately:

Title page (with author details): This should include the title, authors' names, affiliations, acknowledgements and any Declaration of Interest statement, and a complete address for the corresponding author including an e-mail address.
Blinded manuscript (no author details): The main body of the paper (including the references, figures, tables and any acknowledgements) should not include any identifying information, such as the authors' names or affiliations.

Use of word processing software
It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - unnumbered sections
Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

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

Material and methods
Provide sufficient detail to allow the work to be reproduced.

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.

Conclusions
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.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual structured abstract is required. The abstract should briefly state the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s).

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Acknowledgements

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.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Image manipulation
Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then the journal will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites). Further information on the preparation of electronic artwork.

Illustration services
Elsevier's Author Services offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style
Text: Indicate references by (consecutive) superscript arabic numerals in the order in which they appear in the text. The numerals are to be used *outside* periods and commas, *inside* colons and semicolons. For further detail and examples you are referred to the AMA Manual of Style, A Guide for Authors and Editors, Tenth Edition, ISBN 0-978-0-19-517633-9.
List: Number the references in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. *J Sci Commun.* 2010;163:51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:

2. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. *Heliyon*. 2018;19:e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
3. Strunk W Jr, White EB. *The Elements of Style.* 4th ed. New York, NY: Longman; 2000.
Reference to a chapter in an edited book:
4. Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, eds. *Introduction to the Electronic Age.* New York, NY: E-Publishing Inc; 2009:281–304.
Reference to a website:
5. Cancer Research UK. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/; 2003 Accessed 13 March 2003.
Reference to a dataset:
[dataset] 6. Oguro, M, Imahiro, S, Saito, S, Nakashizuka, T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. https://doi.org/10.17632/xwj98nb39r.1.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material
Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data
This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

AFTER ACCEPTANCE

Proofs

One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or a link will be provided in the e-mail so that authors can download the files themselves. To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Elsevier now provides authors with PDF proofs which can be annotated; for this you will need to download the free Adobe Reader, version 9 (or higher). Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site.
If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and scan the pages and return via e-mail. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. It is important to ensure that all corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.