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VICTOR HUGO DE SOUZA RIBEIRO

A RIGIDEZ DO RETO FEMORAL E VASTO LATERAL AVALIADO PELA ELASTOGRAFIA EM INDIVÍDUOS SAUDÁVEIS, É INFLUENCIADO PELO COMPRIMENTO MUSCULAR?

DISSERTAÇÃO DE MESTRADO

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Orientador: Prof. Dr. João Luiz Quagliotti Durigan

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA REABILITAÇÃO

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DEDICATION

I dedicate this dissertation to all my family, and my loving son João Bernardo.

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indicate comparisons between depht groups. Significant differences ($p < 0.05$) are represented by an asterisk (*). 95% CI = Confidence interval; KPa: shear wave; m/s: shear wave velocity.

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LIST OF ABBREVIATIONS

RF: rectus femoris

VL: vastus lateralis

VM: Vastus medialis

VI: vastus intermedium

SUP60: Supine with joint angle in 60°

SUP20: Supine with joint angle in 20°

SIT60: Sitting with joint angle in 60°

SIT20: Sitting with joint angle in 20°

KPa: shear wave elasticity

m/S: shear wave velocity

BMI: body mass index

IPAQ: International Physical Activity Questionnaire

ICC: intraclass correlation coefficient

UP: Upper

INT: Intermediate

MVIC: maximum voluntary isometric contraction

ANOVA: Analysis of variance

RESUMO

A rigidez muscular desempenha um papel crítico na função e no desempenho muscular, afetando fatores como fadiga, atividade metabólica e respostas eletromiográficas. Este estudo teve como objetivo investigar a rigidez dos músculos reto femoral (RF) e vasto lateral (VL) em diferentes ângulos articulares (60° e 20° de flexão do joelho) e posições do corpo (supino e sentado) utilizando Elastografia. Trinta participantes saudáveis foram incluídos em um estudo randomizado cruzado, com idade de 21,98 ± 2,92 anos, altura de 1,70 ± 0,10 metros e peso de 62,35 ± 13,73 quilos. A rigidez muscular foi avaliada utilizando a elasticidade da onda de cisalhamento (kPa) e a velocidade da onda de cisalhamento (m/s), com medidas realizadas nas regiões superficial, intermediária e profunda dos músculos RF e VL. Os resultados indicaram valores de rigidez significativamente maiores no SUP60 (em decúbito dorsal com joelho a uma flexão de 60°) ($p<0,001$) em comparação com outras posições para os músculos RF e VL, medidos pelas variáveis kPa e m/s. A rigidez foi consistentemente maior nas regiões superficiais ($p<0,001$) dos músculos em comparação às regiões intermediárias e profundas em todas as posições. As análises estatísticas revelaram interações significativas entre posição e tipo muscular, bem como entre posição e profundidade, destacando o impacto matizado do ângulo articular e posição corporal na rigidez muscular. Nossos achados de Elastografia indicam que tanto o reto femoral, quanto o vasto lateral apresentam maior rigidez quando posicionadas em SUP60 em comparação com SUP20, SIT60 e SIT20. Além disso, observamos maior rigidez nas regiões superiores desses músculos em todas as posições testadas, em contraste com as regiões intermediárias e profundas.

Palavras-chave: Rigidez muscular, elastografia, ângulo articular, quadríceps.

ABSTRACT

Muscle stiffness plays a critical role in muscle function and performance, affecting factors such as fatigue, metabolic activity, and electromyographic responses. This study aimed to investigate the stiffness of the rectus femoris (RF) and vastus lateralis (VL) muscles at different joint angles (60° and 20° of knee flexion) and body positions (supine and sitting) using elastography. Thirty healthy participants were included in a crossover, randomized trial, with an age of 21.98 ± 2.92 years, height of 1.70 ± 0.10 meters, and weight of 62.35 ± 13.73 kilograms. Muscle stiffness was assessed using shear wave elasticity (kPa) and shear wave velocity (m/s), with measurements taken at superficial, intermediate, and deep regions of the RF and VL muscles. Results indicated significantly higher stiffness values in the SUP60 (supine with knee at 60° flexion) (p<0,001) position compared to other positions for both RF and VL muscles, as measured by both kPa and m/s variables. Stiffness was consistently higher in the superficial regions (p<0,001) of the muscles compared to intermediate and deep regions across all positions. Statistical analyses revealed significant interactions between position and muscle type, as well as between position and depth, highlighting the nuanced impact of joint angle and body position on muscle stiffness. Our elastography findings indicate that both the rectus femoris (RF) and vastus lateralis (VL) exhibit greater stiffness when positioned at SUP60 compared to SUP20, SIT60, and SIT20. Additionally, we observed higher stiffness in the upper regions of these muscles across all tested positions, in contrast to the intermediate and deep regions.

Keywords: Muscle stiffness, elastography, joint angle, quadriceps.

1. INTRODUCTION

Muscle length is a key factor influencing various adaptations in muscle fibers, which may relate to fatigue (Cavalcante et al., 2024), metabolic activity (Al-Mulla, Sepúlveda, Colley., 2011), electromyographic activity (Lanza et al., 2017), and clinical protocols in rehabilitation (de Sousa et al., 2023), among other factors. Numerous studies have examined different muscle lengths under various conditions to observe muscle adaptations, muscle capacity and muscle archiving. We observed in the literature that studies bring to us that a relationship between stretching of the musculoskeletal (Bastijns et al., 2020), however the studies differ about this relation. Kato et al., (2010) show that muscle stiffness is not affected by stretching, contradicting the findings (Blazevich et al., 2009; Morse et al., 2008) that reduce muscle stiffness depending on stretching. Longer lengths tend to have higher levels of KPa (stiffness) compared to shorter lengths in the gastrocnemius muscle (Freitas et al., 2015). The reduction of stiffness over a static relaxation protocol is higher for longer lengths in this study. Another finding of this study is that the Kpa presents a greater significant difference close to the maximal dorsiflexion, compared with reduced articular degrees

Muscle stiffness analysis can reliably be obtained with Elastography using ultrasound imaging in a passive assessment (Gennisson et al., 2010; Liu et al., 2019). Elastography has the advantage of assessing muscle stiffness in different muscle positions without requiring participants to perform muscle contractions (Eby et al., 2015). Some active methods to assess muscle stiffness, such as Young's modulus, evaluate stiffness based on participants' physical efforts (Bravo-Sánchez et al., 2021), which can be unfeasible for individuals with impairments or impossibility to perform a muscle contraction, perhaps, the better way to evaluate this people is using passive methods (Lee et al., 2017; Tas et al., 2018). During stiffness evaluation using elastography, two key variables are typically observed: kilopascals (KPa) and shear wave propagation speed. These variables are interconnected, as the propagation speed is directly dependent on the shear wave properties (Albano et al., 2024; Nicholls et al., 2020).

The quadriceps femoris musculature primarily generates knee extensor torque. Each constituent of the quadriceps—comprising monoarticular muscles: Vastus medialis (VM), vastus lateralis (VL) and vastus intermedium (VI) and a biarticular muscle (Rectus femoris (RF))—experiences changes in force production with alterations in hip and knee joint angles during knee extension (Pincívero et al., 2004). The fascia that surrounds the quadriceps muscles can transmit tension through the joints, affecting muscle stiffness (Schleip et al., 2012). The joint angle significantly influences torque production, with an "optimal" length for force production around 60 degrees of knee flexion (Cavalcante et al., 2023), where 0 degrees represents complete knee extension. In various protocols, comparing supine and sitting hip positions, the strength production of the quadriceps femoris may either decrease or remain unchanged in the supine position across multiple knee joint angles ranging from 20 to 90 degrees (Mafuilletti et al., 2003). Although knee flexion around 60 degrees favors force production, it also increases compression in knee structures. Therefore, investigating muscle stiffness in different positions is necessary to understand the relationship between strength production capacity and muscle stiffness. Few studies have examined muscle length and stiffness, especially in the quadriceps, considering hip and knee joint positioning. Chernak et al., (2013) investigated the relationship between muscle length and stiffness from the speed of the shear wave in the gastrocnemius, manipulating knee joint positioning through active and passive dorsiflexions. They observed greater stiffness indicated by velocity during the most dorsiflexed moments in passive movement, particularly in volunteers with the knee extended compared to those with it more flexed. Therefore, our objective is to investigate the stiffness of the rectus femoris and vastus lateralis at different lengths to observe stiffness behavior in a biarticular and a monoarticular muscle of the quadriceps femoris. We hypothesized that higher stiffness would be observed in the RF and VL muscles in the SUP60 position, as indicated by both the KPa and m/s variables. This increased stiffness would likely be more pronounced in the upper region compared to the others.

2. LITERATURE REVIEW

2.1 Relation between muscle length and force generation capacity

The quadriceps femoris is one of the most studied muscles in the literature, given its importance for locomotion and sports gestures such as running, jumping and landing. The quadriceps femoris is formed by four muscular bellies (constituents), these being: rectus femoris, vast medial, vast intermediate and vast lateral. The rectus femoris presents its origin located in the anterior-inferior iliac spine, the vast lateral in the lateral greater trochanter, the vast medial in the femoral neck and vast intermediate in the femoral body. These muscles form the tendon of the quadriceps femoris, with insertion in the patella, but their tendon fascicles have different orientations to offer resistance in different directions, Moreover, the patella fits in the trochlear groove of the femur acting as an anchor for the quadriceps femoris and the patellar tendon (Depoujari et al., 2021). The femoral quadriceps is primarily responsible for the mechanism of knee extension (Olewnik et al., 2021). For this reason, it is widely explored in the scientific literature, in order to provide better bases for evaluation and therapies in clinical practice. By sharing the same distal insertion, the constituents of the quadriceps femoris are likely to suffer changes due to the change of the knee joint position. The study by Cavalcante et al., (2021) found that knee joint angle positioning interferes with different aspects of the components of the quadriceps femoris, such as torque, stiffness and stretching. The joint torque is a reflection of the structural myotendinous capacities of the volunteer. Muscle length is intrinsically related to the strength production capacity of the muscle fiber (de Sousa et al., 2023), so depending on the muscle length present during a protocol, be it isometric (Oranchuk et al., 2019), isokinetic (Marušič et al., 2020; Wan et al., 2017) evoked (Cavalcante et al., 2023) or voluntary, we will have different outcomes. Muscle length has been extensively investigated in the literature, under different conditions. Many studies investigated different muscle lengths during an electrostimulation protocol. A recent meta-analysis shows that muscle length affects torque during a neuromuscular electrical stimulation protocol. This study made an interesting distinction, grouping the studies by the degree of positioning of the knee joint, during the protocol, in order to separate the lengths into groups: very short, short, great, long and very long. These data related to knee extension bring that: 1- Optimal lengths presents a greater extensor torque when compared to a muscular length very short, short and long. 2- A long length has a higher extensor torque when compared to very short. 3- A long length has a higher extensor torque when compared to short. On the other hand, many studies also investigate the influence of femoral quadriceps muscle length during a voluntary contraction. Scott et al compared the force of a voluntary knee extensor torque in order to investigate which joint positioning would generate a higher torque. The authors compared 3 degrees of the knee joint, 30°; 60°; and 90°; respectively. They observed that the positions in which the quadriceps was more elongated (90° and 60°) produced a higher torque when compared to the position in which the quadriceps was more shortened. Garnier et al., (2021) investigated the influence of hip and knee angle during an isometric maximal voluntary contraction, comparing flexed knee (110°, considering 0° full knee extension) with extended knee (20°), flexed hip (60°) and extended hip (0°) found that: torque was higher when the knee was flexed (greater muscle length), compared to extended knee (shorter muscle length). It also noted that hip positioning was indifferent during maximal isometric voluntary contraction. Cavalcante eat al., (2021) and Bampouras et al., (2017) also corroborate this finding, bringing in their studies that torque is indifferent to the influence of the hip (lying and sitting). The study of Cavalcante et al., (2021) goes even further, bringing that in fact what will determine the power production capacity of the quadriceps, is not the hip positioning, but the knee. These findings corroborate with ancient studies, which show that there is a length-strength relationship (Gordon 1966). Other studies focus on the investigation of the muscular architecture observing the length of it. The architecture of the muscle is reflected in the individual’s torque production capacity. The stiffness of the tendon-aponeurosis complex (Massey et al., 2015), muscle thickness (Blazevich et al., 2006), fascicular length, penation angle (Timmins et al., 2016), neural activation (Maffiuletti et al., 2003) and stiffness (Kubo 2006; Massey 2015) mechanisms of muscle architecture that will reflect on a better tendon muscle capacity. Thus, changes in the angle of the joint, as far as it is concerned, can alter the functionality of the muscle (Kubo 2006; Lanza et al., 2017; Pearson et al.,2017). Therefore, the study of muscle variables affects the action of the system as a whole.

2.2 Muscle stiffness

Muscle stiffness is the combination of the active tension produced through contraction, and passive tension produced by the connective tissue (Eby et al., 2013; Hug et al., 2015), being defined as the compression-deformation ratio (Ikezoe et al., 2012; Creze et al., 2018). This deformation can be created from an external action, often being compression, or an intrinsic tissue deformation, dependent on tissue properties (Drakonaki et al.,2012; Brandenburg et al., 2014). Over time it is possible to observe several methods available for the analysis of passive musculoskeletal stiffness. Passive stretching test is a common technique used to measure passive stiffness of muscles and joints. Due to the measurement of resistance offered by the elongated muscle (Magnusson 1966). We can observe the measurement of stiffness through dynamometry, which will measure the passive resistance during the stretching or movement of the joints (Aagard 2000). Another means of evaluating this stiffness is through magnetic resonance imaging, which will use magnetic fields and radio waves to create detailed images of the surrounding muscles and tissues, assessing passive muscle stiffness through visualization of internal muscle structures. (Dixon 1992; Rigleb et al., 2007).

2.3 Relation Stiffness- Hip/Knee joint

Stiffness in the muscular belly of knee extensors, such as the muscles of the quadriceps group, is determined by several structures and factors: 1) Muscle Fibers: The intrinsic properties of muscle fibers, including their type (slow vs. fast contraction fibers) significantly influence muscle stiffness (Lieber, Fridén, 2000; Schiaffino, Reggiani; 2011). 2)Connective tissue: The endomysium, perimysium and epimysium, which involve muscle fibers, fascicles and all muscle, respectively, contribute to passive stiffness (Purslow et al., 2002; Gillies, Lieber; 2011). 3)Titin: Titin, a giant protein extending half the length of a sarcomere, plays a key role in passive muscle stiffness by providing elasticity (Granzier, Labeit, 2004; Linke et al., 2008). 4)Fascia: The fascia surrounding the muscles, particularly the deep fascia like the fascia lata, contributes to general muscle stiffness (Stecco, et al., 2015; Schleip et al., 2012). 5) Neural Factors: Muscle tone, regulated by the nervous system, affects stiffness. Increased muscle tone due to increased neural activation may lead to greater stiffness (Kandel et al., 2000; Butler, Moseley 2003). 5)Viscoelastic properties: The viscoelastic properties of muscle tissues, which include their deformation capacity and return to their original shape, impact stiffness (Magid, Law 1985; Fung et al., 1993). The joint positions of the hip and knee significantly influence the structures that determine stiffness in the muscular belly of the knee extensors. Thus, the relationship between muscle length-tension, passive tension, neural factors and fascial interactions are key aspects of this influence. The position of the hip and knee affects the length of the quadriceps muscles. For example, hip flexion and knee extension lengthen the rectum due to its biarticular nature (Gordon et al., 1966; Lieber, Fridén, 2000). When the hip and knee are positioned to lengthen the quadriceps (hip extension and knee flexion), passive tension increases due to the stretching of connective tissues and Titin protein (Magnusson et al., 1996; Granzier, Labeit, 2004). We can observe in the literature that joint position influences neural activation through mechanisms such as muscle spindles and Golgi’s tendon organs, adjusting muscle tone and stiffness (Kandel et al., 2000; Enoka et al., 2008). The fascia that surrounds the quadriceps muscles can transmit tension through the joints, affecting muscle stiffness, so changes in hip and knee positions alter the tension in these fascial structures (Stecco, 2015; Schleip et al., 2012). The fascial connections of the rectus femoris and vastus lateral muscles are also crucial for the biomechanics of the knee and hip joints. We can also observe that the fascia of the rectus femoris integrates with the quadriceps tendon and the patellar ligament, providing stability and support to the knee joint (Stecco, et al., 2015; Langevin, Sherman; 2007). In the hip, the femoral rectum fascia is continuous with the iliotibial band (ITB) and the fascia lata tensor (TFL), influencing the hip movement and stability (Schleip et al., 2012; Barker et al., 2014). The fascia of the wide side integrates with the ABI and the lateral retinaculum of the knee, providing lateral stability to the patella and knee joint (Standring, 2015; Fairclough et al., 2006). In the hip, we can observe that the fascia of the lateral vast is interconnected with the gluteal fascia, influencing abduction and hip stability (Willard et al., 2012; Huijing, Jaspers;

2005).

2.4 Shear Wave Elastography (SWE)

Lately we have observed that many studies have been dedicated to the study of muscle stiffness, from Shear wave elastography (Davis et al., 2019), and this technique has advantages, among others because: 1) direct evaluation of elasticity; 2) independence of the compression that the tissue undergoes; 3) Quantitative data. The acquisition of the stiffness data of Shear wave elastography, is performed from an acoustic radiation force impulse (ARFI). This technique generates a high-intensity impulse beam, coming from the transducer, to the tissue. After the impulse beam, the transducer measures tissue displacement, instantly generating a qualitative map of stiffness in a B-mode image (Bastijns et al.,2020) . After mapping the tissue stiffness, a measurement, related to tissue tension ratio (ROI) is provided. The ROI is totally dependent on the operator, and therefore more qualitative. Soon Shear wave elastography provides us with both quantitative and qualitative data. The data are expressed as shear (KPa) and/or shear wave velocity (cm/s). Some factors can quality of acquisition of these data, and some main care to be taken during image acquisition. First caution: Transducer pressure. Carpenter et al., (2015) brings that depending on the pressure maintained in the transducer, there may be changes in the data. For example, Kot et al., (2012) observed that 3 different pressure levels provide data difference, where increased pressure suggests increased muscle stiffness of the participant. Second: Position of the transducer. Studies such as (Cortez 2016 et al., Alfuraih et al., 2017) suggest a difference in acquisition speed according to the aponeurosis approach. Third: differences between systems and devices. There is low reliability between measurements performed by different equipment (Alfuraih 2017 et al.,; Franchi-Abella et al., 2013).

2.5 Applicability of the SWE

Providing quantitative and qualitative muscle data, elastography allows its application both in a clinical setting and in a research setting. We can observe that many disorders are based on the condition and structural changes of the muscles, so elastography can allow the finding, and location of certain pathologies. In addition to identifying the pathology, elastography can act in order to monitor the evolution during an intervention protocol, or treatment. Due et al., (2016) evaluated the ability of elastography, through Shear wave, to assess stiffness in patients with Parkinson’s disease, since muscle stiffness is one of the symptoms of Parkinson’s. These authors concluded that SWE can be used to assess muscle stiffness. Ding et al., (2021) goes according to the findings, indicating that SWE can be a way of assessing stiffness in patients with Parkinson's disease. Another neuromuscular disorder that has been investigated with the use of SWE is Stroke. Stroke will cause greater muscle stiffness on the affected side compared to the unaffected side. Eby et al., (2016) proposed to evaluate the potential of SWE in individuals with muscle stiffness after Stroke. The authors observed that SWE is promising in the evaluation of muscle stiffness in post-Stroke individuals. In addition to evaluating stiffness, in post-Stroke individuals, we also observed that it can act following the intervention in these individuals (Gao 2019). We also observed other conditions present in the literature that can be evaluated or diagnosed through SWE. Cerebaral palsy (Branderburg et al., 2016; Lee 2016; Vola et al., 2018), Duchenne muscular dystrophy (Larcourpaille et al., 2015; Pichiecchio et al., 2018), sarcopenia (Alfuraih et al., 2019b; Bastijns et al., 2019), chronic neck pain (Tas et al., 2018), among others. It is possible to observe in literature studies are dedicated to investigate the influence of sex on musculoskeletal stiffness, in order to observe the difference between groups. Some studies show that women have greater stiffness than men (Lima et al., 2018; Chen et al., 2017), others show higher values in men when compared to women (Agypong-Badu et al., 2016; Wang et al.,2017), and others do not find significant difference between groups. Another well studied relationship is stiffness in aging, however the data are still inconclusive. Studies report in favor of greater stiffness in younger individuals (Eby et al., 2015; Saito et al., 2019), but studies have been seen in which older individuals have greater stiffness (Akagi et al., 2015), while we observed studies that found no difference between groups (Ikezoe et al.,2012). Among some unanswered questions, such as those mentioned above, a question is still recurrent regarding the positioning of the subject during the acquisition of muscle stiffness via SWE. Depending on muscle length, we can increase or decrease muscle tension, and consequently, change muscle stiffness values. Davis et al., (2019) reports that a small increase in flexion can change values during data acquisition. It is possible to observe in the literature that few studies pay attention to the importance of muscle length during a protocol of stiffness analysis, in healthy or compromised individuals. Thus, this study aims to elucidate this relationship, investigating the association of these variables in the femoral quadriceps.

3. JUSTIFICATIVE

Recently many studies have evaluated the passive muscular stiffness of the femoral quadriceps using elastography. However, these studies do not focus on the joint positioning of the hip and knee, since the quadriceps has monoarticular and biarticular constituents. Previous studies show that joint positioning interferes with some structures, however the relationship between passive stiffness through elastography and angle of knee and hip is not yet clear.

4. OBJECTIVE

Our objective is to investigate the stiffness of the rectus femoris and vastus lateralis at different lengths to observe stiffness behavior in a biarticular and a monoarticular muscle of the quadriceps femoris.

5. HYPOTHESIS

We hypothesized that higher stiffness would be observed in the RF and VL muscles in the SUP60 position, as indicated by both the KPa and m/s variables. This increased stiffness would likely be more pronounced in the upper region compared to the others.

6. MATERIAS AND METHODS

6.1 Experimental design

This study is a crossover, experimental, and randomized trial conducted at the Musculotendineal Plasticity Laboratory (LaPlasT) of the Faculty of Ceilândia, University of Brasília. The project was approved by the Research Ethics Committee of the University of Brasília/Faculty of Ceilândia, in accordance with Resolution 510/16 of the National Health Council (CAAE: 68446223.2.0000.8093). Following approval by the committee, the research protocol was registered on ClinicalTrials.gov (68446223.2.0000.80). Participants were thoroughly informed about the purposes, benefits, and risks of the study at all stages. Their participation was contingent upon signing the informed consent form, ensuring their voluntary and informed entry into the study.

6.2 Participants

We included 30 participants (13 men and 17 women) with an average age of 21.98 ± 2.92 years, height of 1.70 ± 0.10 meters, and weight of 62.35 ± 13.73 kilograms. All participants were healthy, with a body mass index (BMI) between 18.5 and 24.9 kg/m² (eutrophic), and had not undergone systematic lower limb strength training in the past six months. Participants were physically active according to the International Physical Activity Questionnaire (IPAQ), engaging in either recreational or sports activities. Exclusion criteria included individuals with edema, dermal injury, limited joint range of motion, deformity or amputation in any part of the lower limbs, history of patellar dislocation, or trauma to the lower limbs or trunk that could compromise results. Additionally, those with conditions affecting musculotendineal morphology or neuromuscular excitability, such as type II diabetes mellitus, familial hypercholesterolemia, neuromuscular disease, or severe cardiopathy, were excluded. Participants with conditions preventing cooperation with procedures, such as cognitive deficits, psychiatric disease, chemical dependence, or behavioral problems (Dudley-Javoroski 2010), were also excluded.

6.3 Randomization and concealment of allocation

In the familiarization session, each participant had their collection randomized by a randomization application, and their allocation was related to the assorted positions randomly by the application. The positions were as follows: SUP60 (lying with the knee at 60°), SUP20 (lying with the knee at 20º), SIT60 (sitting with the knee at 60°) and SIT20 (sitting with the knee at 20°).

6.4 Blinding

The participants were blinded to the hypotheses of the study and to the numerical values of the joint angles used. They were not informed which position was expected to result in greater muscle stiffness. However, due to the nature of each position, the evaluator could not be blinded.

6.5 Elastography

Muscle stiffness in the RF and (VL was evaluated using shear wave elasticity (kPa) and shear wave velocity (m/s). Musculoskeletal configurations ranging from 0-300 kPa were selected using the ACUSON Redwood Ultrasound System (Siemens, USA). Specifically, the lateral compartment of the RF and the VL were visualized at 50% and 60% of the thigh length, respectively, measured from the anterior-superior iliac spine to the base of the patella (Cavalcante et al., 2021a). From B-mode ultrasound images obtained with a linear transducer (10 L4), ROIs were delineated in a circular format. Thirty ROIs were manually measured: 10 in the superficial region of each muscle (superior part in selection box), 10 in the deep region (deep part in selection box), and 10 in the intermediate region between the superficial and deep regions (medium part in selection box). Muscle tissue stiffness was determined by averaging the shear modulus velocity from these 30 ROIs apply in each depth per image. The stiffness of each muscle was calculated based on the mean shear modulus from three obtained images. This mean shear modulus represented the stiffness of the quadriceps femoris components. During the ultrasound examination, the transducer surface was covered with water-soluble transmission gel to ensure acoustic coupling without applying additional pressure on the skin, thereby preventing overestimation of tissue stiffness.

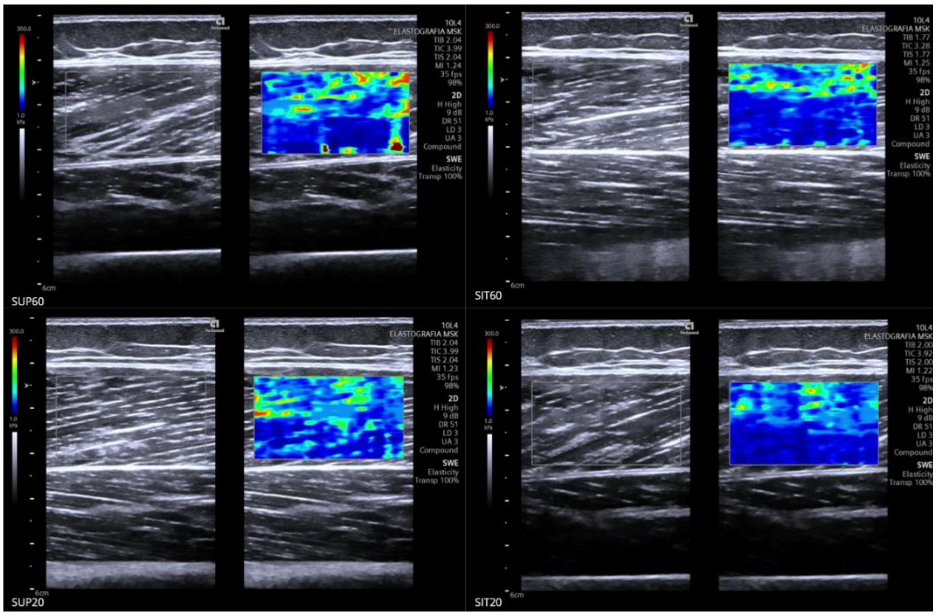

Figure 1. Visualization of the VL muscle of a participant in all positions. Insert the sealing box between the aponeuroses, in order to fill the ROI s in the upper, medial and lower part of this box.

All elastography evaluations were performed by the same evaluator, VHSR. The evaluation environment was maintained at temperatures between 23-25ºC to prevent fluctuations in tissue stiffness (Ando & Suzuki, 2019). Three ultrasound images of each tissue (superficial and deep fascia of each muscle, RF and VL) were acquired with the participant in a resting position. Although the acquisition time of the images does not affect stiffness values, a stabilization period for the elastography map is necessary (Lima 2018). Therefore, images were captured after a 10-second delay to ensure the map's stabilization (Lima 2017).

6.6 Study design

Participants performed familiarization with the assessments, which included anthropometric measurements (height and body mass), randomization of the order of joint positioning, and verification of eligibility criteria. The following positions were evaluated:

supine with the knee at 60º flexion (SUP60), seated with the knee at 60º flexion (SIT60), supine with the knee at 20º flexion (SUP20), and seated with the knee at 20º flexion (SIT20). These knee angles (60º and 20º) were assessed with participants in sitting (hip flexion; 85º) and supine (hip extension; 0º) positions. The rectus femoris (RF) and vastus lateralis (VL) muscles were selected to observe the stiffness behavior of two muscles within the same group, with one being biarticular and the other monoarticular. During anthropometric assessment, participants were barefoot and wore light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ), which evaluates the frequency and duration of physical activities over the past week (Scholes et al., 2016). Following familiarization, four sessions were conducted to evaluate stiffness using elastography. Each session tested one of the four study positions and was separated by 72 hours.

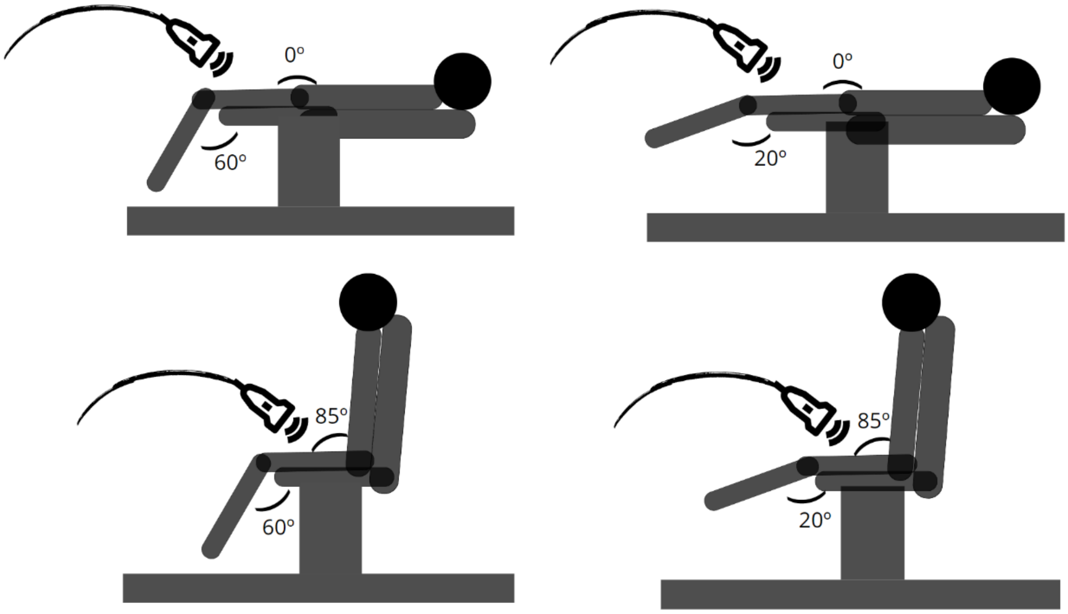

Figure 2. Analysis positioning. Bench press with knee at 60º of flexion (SUP60), sitting with knee at 60º (SIT60), bench press with knee at 20º (SUP20) and sitting with knee at 20º (SIT20). The positions were randomized in the familiarization session.

Figure 3. Data collection. Dynamometer axis aligned with the center of the knee joint, for evaluation of stiffness in the RF during the SUP20 position.

6.7 Statistical analysis

The results were reported through measures of central tendency and dispersion, appropriate inference tests, tables and graphs. The normality of the data was verified with the Shapiro-Wilk test. A three-way ANOVA [“position” (4 levels: SUP60, SUP20, SIT60 and SIT20) by “muscle” (2 levels: RF and VL) by “depth” (3 levels: upper, intermediate and deep)] was applied to verify changes in Kpa and m/S. When a significant difference was detected, we applied the post-hoc Tukey test. The significance threshold is defined in $\alpha < 0.05$ for all procedures. All statistical analyses was performed using the software Statistica 23.0 (STATSOFT Inc., Tulsa, Oklahoma, EUA) and for graphical construction the software Graphpad Prism 8.3.0 (San Diego, California, USA). To determine the reliability of the measurements, we calculated the intraclass correlation coefficient (ICC). Reliability was classified as low (< 0.5), moderate (0.5 - 0.75), good (> 0.75 - 0.9) and excellent (> 0.9) (Koo 2016).

7. RESULTS

7.1 Reliability of elastography

After intra-evaluator ICC analysis, good reliability was observed for RF SUP60 KPa (0.90), RF SUP20 KPa (0.88), RF SIT60 KPa (0.80), and RF SIT20 KPa (0.87). Similarly, good reliability was found for RF SUP60 m/s (0.90), RF SUP20 m/s (0.82), RF SIT60 m/s (0.80), and RF SIT20 m/s (0.80). For VL, excellent reliability was observed for SUP60 KPa (0.92), SUP20 KPa (0.92), and SIT60 KPa (0.92), and good reliability for SIT20 KPa (0.80). Similarly, excellent reliability was found for SUP60 m/s (0.92) and SUP20 m/s (0.90), and good reliability for SIT60 m/s (0.88) and SIT20 m/s (0.82). Comparing position and muscles

There was a significant difference in position (p<0.001), muscle (p=0.001), depth (p<0.001) when analyzing KPa data. There was a significant difference in position (p<0.001), muscle (p=0.001), depth (p<0.001) when analyzing data related to m/s.

7.2 Position x muscle

To assess KPa, the main effect of position and muscle (p < 0.001) (Table 1- Suplementary table) indicated that the SUP60 group exhibited significantly higher KPa values compared to the other groups for RF (estimated marginal mean [95% confidence interval]: SUP60 = 12.41 [11.30 – 13.52] %, SUP20 = 7.76 [7.14 – 8.39] %, SIT60 = 7.87 [7.33 – 8.41] %, SIT20 = 6.78 [6.21 – 7.35] %) and for VL (estimated marginal mean [95% confidence interval]: SUP60 = 9.63 [8.67 – 10.59] %, SUP20 = 7.22 [6.56 – 7.89] %, SIT60 = 8.30 [7.57 – 9.02] %, SIT20 = 6.52 [5.96 – 7.09] %) (figure 4). In terms of m/s, the main effect of position and muscle (p < 0.001) similarly showed that the SUP60 group had higher m/s values compared to the other groups for RF (estimated marginal mean [95% confidence interval]: SUP60 = 1.97 [1.88 – 2.07] %, SUP20 = 1.57 [1.51 – 1.63] %, SIT60 = 1.64 [1.55 – 1.73] %, SIT20 = 1.46 [1.41 – 1.52] %) and for VL (estimated marginal mean [95% confidence interval]: SUP60 = 1.74 [1.66 – 1.83] %, SUP20 = 1.51 [1.45 – 1.58] %, SIT60 = 1.66 [1.58 – 1.73] %, SIT20 = 1.44 [1.38 – 1.50] %). There was a significant interaction between position x muscle for variable Kpa (F = 14.15, P < 0.001, power = 0.99, ηρ2 = 0.32). KPa was significantly higher in SUP60 compared to SUP20 (p<0.001), SIT60(p<0.001) and SIT20(p<0.001) in RF. The KPa in VL was significantly higher in SUP60 compared to SUP20 (p<0.001), SIT60 (p=0.01) and SIT20 (p<0.001). RF KPa was significantly higher than VL in the SUP60 position (p<0.001), however there was no significant difference when comparing the muscles in the SUP20 (p=0.82), SIT60 (p=0.94) and SIT20 (p=0.99) positions. There was a significant interaction between position x muscle for variable m/S (F = 9.13, P < 0.001, power = 0.99, ηρ2 = 0.23). The m/S was significantly higher in SUP60 compared to SUP20 (p<0.001), SIT60 (p<0.001) and SIT20 (p<0.001) in RF. The m/S in VL was significantly higher in SUP60 compared to SUP20 (p<0.001), and SIT20 (p<0.001), and there was no significant difference in SUP60 (p=0.20). RF m/S was significantly higher than VL in the SUP60 position (p<0.001), however there was no significant difference when comparing the muscles in the SUP20 (p=0.76), SIT60 (p=0.99) and SIT20 (p=0.99) positions.

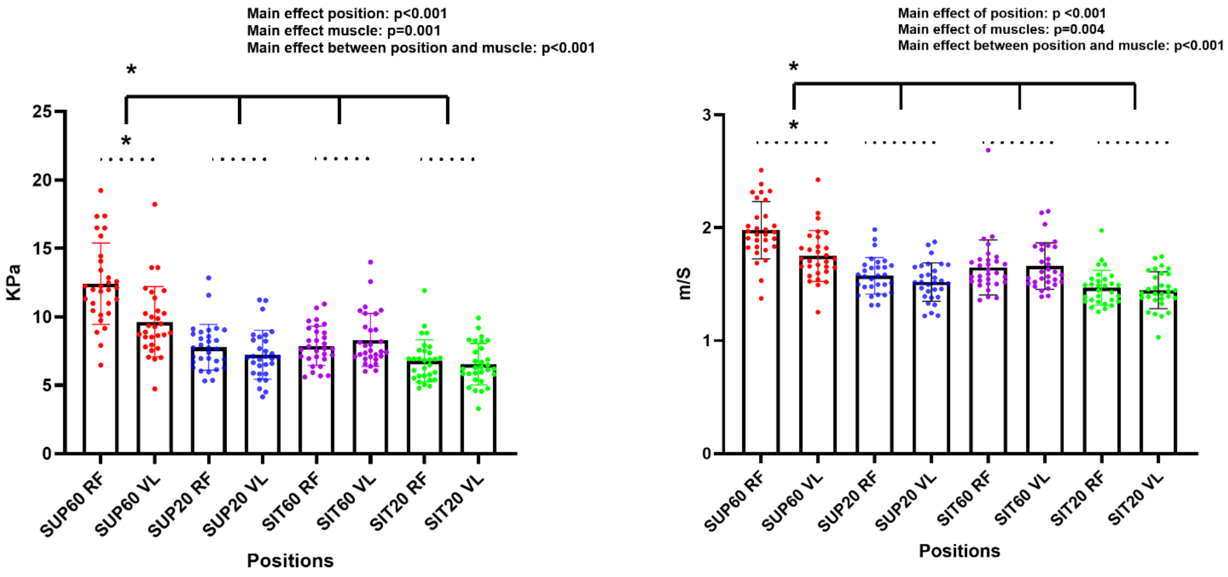

Figure 4: Bar graphs. (mean and 95% CI) comparing muscle ultrasonography variables: KPa (A), m/S (B) m/S, obtained in the SUP60 UP, SUP60 INT, SUP60 DEEP, SUP20 UP, SUP20 INT, SUP20 DEEP, SIT60 UP, SIT60 INT, SIT60 DEEP and SIT20 UP, SIT20 INT, SIT20 DEEP. Black dotted loops indicate comparisons between position groups. Traced line indicate comparisons between depht groups. Significant differences (p <0.05) are represented by an asterisk (*). 95% CI = Confidence interval; KPa: shear wave; m/s: shear wave velocity; SUP60: supine with knee 60; SUP20: supine with knee 20; SIT60: siting with knee 60; SIT20: sitting with knee 20; RF: Rectus femoris; VL: Vastus lateralis; UP: Upper; INT: Intermediate.

Table 1. Kpa an m/S in different muscles grouped by joint angle

Rectus femoris	**SUP60**	**SUP20**	**SIT60**	**SIT20**
KPa	12.41	7.76	7.87	6.78
	(11.30, 13.52)	(7.14, 8.39)	(7.33, 8.41)	(6.21, 7.35)
m/S	1.97	1.57	1.64	1.46
	(1.88, 2.07)	(1.66, 1.83)	(1.55, 1.73)	(1.41, 1.52)
Vastus lateralis				
KPa	9.63	7.22	8.30	6.52
	(8.67, 10.59)	(6.56, 7.89)	(7.57, 9.02)	(5.96, 7.09)
m/S	1.74	1.51	1.66	1.44
	(1.66, 1.83)	(1.45, 1.58)	(1.58, 1.73)	(1.38, 1.50)

Values are reported as estimated marginal means from the models (95% confidence interval).

7.3 Comparing position and depth

The main effect of position and depth on kPa values (Table II- Suplementary table) (p = 0.003) indicated significantly higher stiffness values at the upper depth across all evaluated positions: SUP60 = 12.52 [11.40 – 13.65] %, SUP20 = 8.98 [7.85 – 10.12] %, SIT60 = 9.06 [8.37 – 9.75] %, and SIT20 = 7.51 [6.55 – 8.48] % (figure 5). Intermediate depth also showed substantial stiffness, albeit lower than the upper depth: SUP60 = 10.49 [9.64 – 11.34] %, SUP20 = 7.00 [6.47 – 7.53] %, SIT60 = 7.68 [7.18 – 8.18] %, and SIT20 = 6.35 [5.85 – 6.84] %. The deep region exhibited the lowest stiffness values: SUP60 = 10.05 [9.25 – 10.84] %, SUP20 = 6.49 [6.15 – 6.83] %, SIT60 = 7.51 [7.07 – 7.96] %, and SIT20 = 6.09 [5.82 – 6.37] %. Similarly, for m/s, the main effect of position and depth (p = 0.003) demonstrated higher velocities at the upper depth: SUP60 = 1.99 [1.89 – 2.08] %, SUP20 = 1.68 [1.58 – 1.77] %, SIT60 = 1.73 [1.67 – 1.80] %, and SIT20 = 1.54 [1.45 – 1.63] %. Intermediate depth also showed considerable velocity, with slightly lower values compared to the upper depth: SUP60 = 1.82 [1.74 – 1.89] %, SUP20 = 1.50 [1.45 – 1.56] %, SIT60 = 1.60 [1.53 – 1.66] %, and SIT20 = 1.42 [1.36 – 1.47] %. The deep region exhibited the lowest velocities: SUP60 = 1.77 [1.70 – 1.85] %, SUP20 = 1.44 [1.41 – 1.48] %, SIT60 = 1.62 [1.52 – 1.72] %, and SIT20 = 1.40 [1.36 – 1.44] %. There was a significant interaction between position x depth for variable Kpa (F = 3.39, P = 0.003, power =0.93, ηρ2 = 0.10). KPa was significantly higher in the upper region compared to Intermediate and deep in all positions (p<0.001). The KPa in the upper region in SUP60 showed significant difference compared to Intermediate ($p < 0.001$) and deep ($p < 0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p = 0.75$). The KPa in the Upper region in SUP20 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.55$). The KPa in the Upper region in SIT60 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.99$). The KPa in the Upper region in SIT20 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.99$). There was a significant interaction between position x depth for variable m/S ($F = 3.10$, $P = 0.006$, power = 0.91, $\eta\rho2 = 0.09$). The m/S was significantly higher in the upper region compared to Intermediate and deep in all positions ($p<0.001$). The m/S in the upper region in SUP60 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.84$). The m/S in the Upper region in SUP20 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.29$). The m/S in the Upper region in SIT60 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.99$). The m/S in the Upper region in SIT20 showed a significant difference compared to Intermediate ($p<0.001$) and deep ($p<0.001$), however there was no significant difference in the comparison between Intermediate and deep ($p=0.99$).

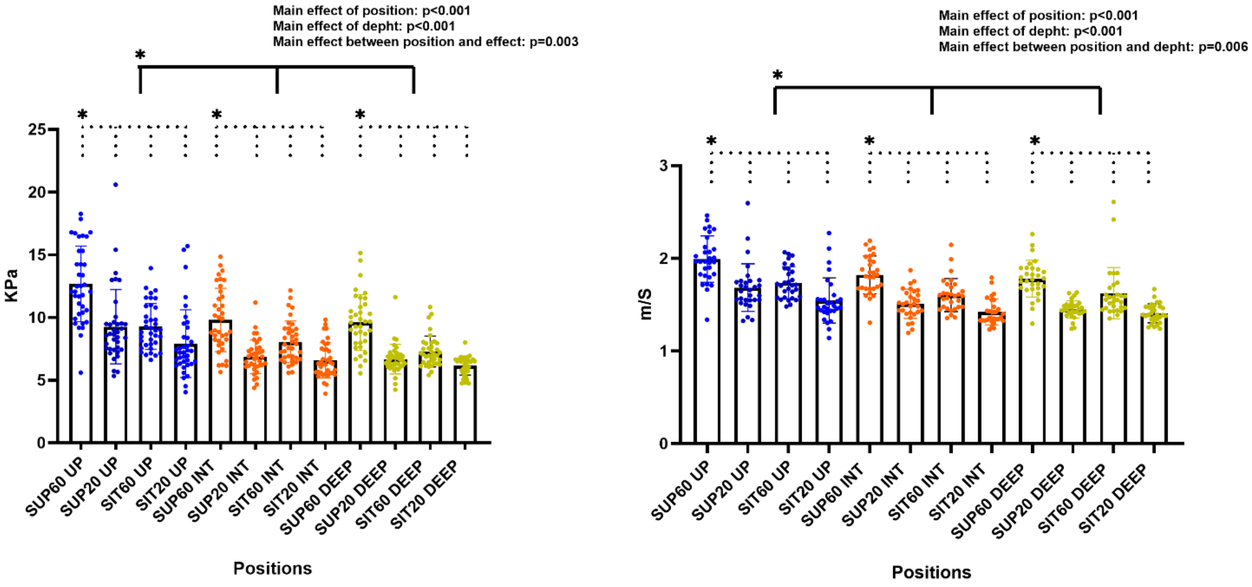

Figure 5: Bar graphs. (mean and 95% CI) comparing muscle ultrasonography variables: KPa (A), m/S (B) m/S, obtained in the SUP60 UP, SUP60 INT, SUP60 DEEP, SUP20 UP, SUP20 INT, SUP20 DEEP, SIT60 UP, SIT60 INT, SIT60 DEEP and SIT20 UP, SIT20 INT, SIT20 DEEP. Black dotted loops indicate comparisons between position groups. Traced line indicate comparisons between depht groups. Significant differences (p <0.05) are represented by an asterisk (*). 95% CI = Confidence interval; KPa: shear wave; m/s: shear wave velocity; SUP60: supine with knee 60; SUP20: supine with knee 20; SIT60: siting with knee 60; SIT20: sitting with knee 20; RF: Rectus femoris; VL: Vastus lateralis; UP: Upper; INT: Intermediate.

Table 2. Kpa and m/S at different depht levels grouped by joint angle

Upper	**SUP60**	**SUP20**	**SIT60**	**SIT20**
KPa	12.52	8.98	9,06	7.51
	(11.40, 13.65)	(7.85, 10.12)	(8.37, 9.75)	(6.55, 8.48)
m/S	1.99	1.68	1.73	1.54
	(1.89, 2.08)	(1.58, 1.77)	(1.67, 1.80)	(1.45, 1.63)
Intermediate				
KPa	10.49	7.00	7.68	6.35
	(9.64, 11.34)	(6.47, 7.53)	(7.18, 8.18)	(5.85, 6.84)
m/S	1.82	1.50	1.60	1.42
	(1.74, 1.89)	(1.45, 1.56)	(1.53, 1.66)	(1.36, 1.47)
Deep	**SUP60**	**SUP20**	**SIT60**	**SIT20**
KPa	10.05	6.49	7.51	6.09
	(9.25, 10.84)	(6.15, 6.83)	(7.07, 7.96)	(5.82, 6.37)
m/S	1.77	1.44	1.62	1.40
	(1.70, 1.85)	(1.41, 1.48)	(1.52, 1.72)	(1.36, 1.44)

Values are reported as estimated marginal means from the models (95% confidence interval).

7.4 **Comparing depth (upper, intermediate, deep) KPa and m/S between groups**

A main effect of depth was observed for kPa (p < 0.001), with higher values in the superficial region (9.35 [8.70 – 10.33] %) compared to both the intermediate (7.88 [7.42 – 8.34] %) and deep (7.54 [7.20 – 7.87] %) regions (figure 6). Similarly, for m/s, a main effect of depth was observed (p < 0.000), characterized by higher values in the superficial region (1.73 [1.66 – 1.81] %) compared to both the intermediate (1.58 [1.53 – 1.63] %) and deep (1.56 [1.51 – 1.61] %) regions. Significant interaction was observed between depht for variable Kpa (F = 35.54, P < 0.001, power =1.00, ηρ2 = 0.55 The Upper KPa region was significantly higher compared to Intermediate and Upper (p<0.001). However, there was no significant difference between Intermediate and deep (p=0.36). Significant interaction was observed between depht for variable m/S (F = 30.19, P < 0.001, power = 1.00, ηρ2 = 0.51). The Upper m/S region was significantly higher compared to Intermediate and deep (p<0.001). However, there was no significant difference between Intermediate and deep (p=0.59).

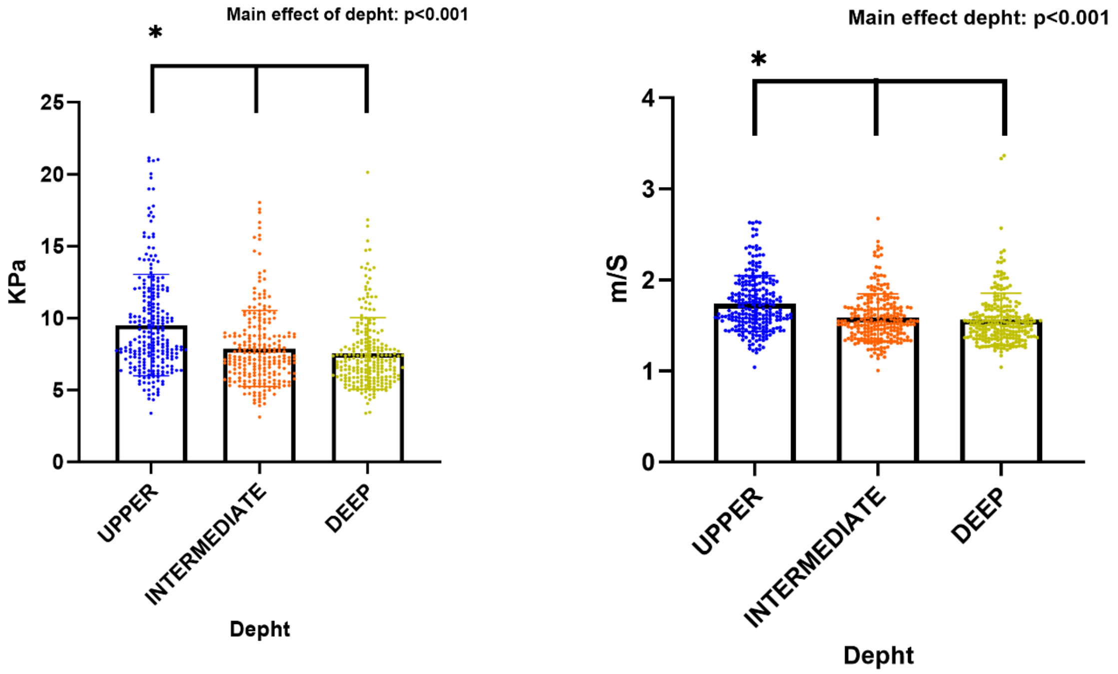

Figure 7: Bar graphs. (mean and 95% CI) comparing muscle ultrasonography variables: KPa (A), m/S (B) m/S, obtained in the upper, intermediate and deep regions. Traced line indicate comparisons between depht groups. Significant differences (p <0.05) are represented by an asterisk (*). 95% CI = Confidence interval; KPa: shear wave; m/s: shear wave velocity.

8. DISCUSSION

This is the first study to evaluate the muscular stiffness of RF and VL through elastography, manipulating the hip and knee joint positioning in healthy adults. Our study identified the following findings: (1) Higher stiffness (KPa and m/s) was observed in the SUP60 position for both RF and VL. (2) Stiffer conditions (KPa and m/s) were consistently found in the superficial regions across all positions compared to the intermediate and deep regions. (3) Greater stiffness (KPa and m/s) was noted in the superficial region compared to both the intermediate and deep regions. Thus, structure architecture and the arrangement of structures in SUP 60 position, generates a greater passive rigidity compared to other positions. The surface regions have a higher stiffness compared to the other regions. Probably because the wave has more difficulty in spreading in deeper regions of the muscle.

Recognizing higher stiffness in specific muscles or positions through research and how the structures will relate, in the future can contributes to clinical practice by guiding diagnosis, treatment planning, functional improvement, and injury prevention. Changes in stiffness can be explained to changes in structure as connective tissues as fascias (Granzier, Labeit, 2004; Stecco et al.,2015), and this can explain the behavior of rectus femoris (biarticular) and vastus lateralis (monoarticular), even hip not influencing the vastus lateralis.

Our data analysis revealed that the SUP60 position exhibited higher stiffness in both KPa and m/s compared to the other positions. This observed difference in stiffness may be attributed to the specific muscle capacity in this joint configuration. Muscle capacity, influenced by the formation of cross-bridges, directly correlates with Young’s modulus and serves as an indicator of strength during isometric contractions (Bastijns et al.,2020). In a similar protocol, but using an active assessment, de Sousa et al., (2023) demonstrated that the Young modulus of the quadriceps femoris is higher when the knee is flexed at 60º compared to 20º during voluntary isometric contractions. Furthermore, their study indicated that the SUP60 position elicited higher maximum voluntary isometric contraction (MVIC) values compared to SUP20, SIT60, and SIT20, suggesting superior force transmission capability in the SUP60 position. Studies by Lui et al., (2019) and Hug et al., (2015) suggest a relationship between muscle strength and elastography. Additionally, Bouillard et al., (2011) proposed a correlation between torque production and kilopascals (KPa) during muscle contraction.

While numerous studies have explored the quadriceps in literature (Ewertsen et al., 2016; Carpenter et al., 2015; Alfuraih et al., 2018; Lacourpaille et al.,2015), no study to date has systematically evaluated muscle stiffness using elastography with varied joint positioning of participants. Most investigations into these muscles typically employ protocols where subjects are positioned supine (Andonian et al.,2016 ; Kot et al., 2021; Alfuraih et al., 2018). However, these studies exhibit significant variability in the specific knee joint angles tested (20º, 30º, and 0º, respectively). Bastijns et al., (2020) highlights in a review one of the primary challenges in elastography as the lack of protocol uniformity and standardization across studies. Dillman et al., (2015), Alfuraih et al., (2017), and Franchi-Abella et al., (2013) have noted that outcomes vary based on the ultrasound system, methodology, and transducer used, complicating direct comparisons between studies. In our study, we observed that the upper region consistently exhibited higher stiffness across all evaluated positions compared to the intermediate and deep regions. This finding is consistent with previous research. For example, Ewertsen et al., (2016) investigated muscles such as the biceps brachii, gastrocnemius, and QF, segmenting their images to analyze stiffness values across different muscle depths. They similarly reported decreasing stiffness values with increasing depth. These findings support our observations regarding variations in muscle stiffness across different regions within the same muscle group.

The reliability of elastography diminishes with increasing muscle depth due to reduced shear wave propagation, as highlighted by Creze et al., (2017). This effect is more pronounced in robust muscles compared to less robust ones. Several factors contribute to the attenuation of wave propagation, including adipose tissue thickness, BMI, muscle depth, fiber type composition (type I and II fibers), bone structure, and underlying pathologies (Bastijns et al., 2020; Drakonaki et al., 2012). Cadaver studies have examined how these tissues affect stiffness measurements, but findings on their impact vary (Yoshitake et al., 2016). Recommendations by Alfuraih et al., (2018) suggest limiting depth to 4 cm for accurate measurements, although applicability may vary depending on population, region, anatomy, and muscle type. In contrast, Carpenter et al., (2015) observed differences in data but did not exceed a depth of 2.5 cm in their measurements. The heterogeneity in study characteristics likely contributes to variations in reported data across different research efforts. Thus, an alternative approach will attempt to standardize the protocol with shear wave elastography (SWE), observing confounding variables such as muscle length, depth of the region of interest (ROI) and adipose tissue. It is important to acknowledge the limitations of this study. Firstly, the results SWE can vary significantly depending on the protocol, evaluator, tissue characteristics, and adipose tissue layer. Variations in the distribution of regions of interest (ROI) can occur, particularly in deeper regions compared to superficial ones, influenced by individual adipose tissue characteristics. Even with participants of similar characteristics, controlling for these layers' interference presents challenges. Additionally, our study involved data collection sessions with seven days between them. This could potentially induce adaptations in the muscles analyzed, reflecting changes in participants' routines over time. Therefore, for future studies employing similar protocols, we recommend conducting data collection sessions on the same day to minimize the influence of external factors as much as possible.

9. CONCLUSION

Our elastography findings indicate that both the rectus femoris (RF) and vastus lateralis (VL) exhibit greater stiffness when positioned at SUP60 compared to SUP20, SIT60, and SIT20. Additionally, we observed higher stiffness in the upper regions of these muscles across all tested positions, in contrast to the intermediate and deep regions.

10. PRACTICAL IMPACTS OF FINDINGS FOR SOCIETY

This study serves as a first step to a greater understanding of this new technology, as well as the observation of the relationships between muscle structures, to in the future enable more complex investigations and understanding as all about these relationships.

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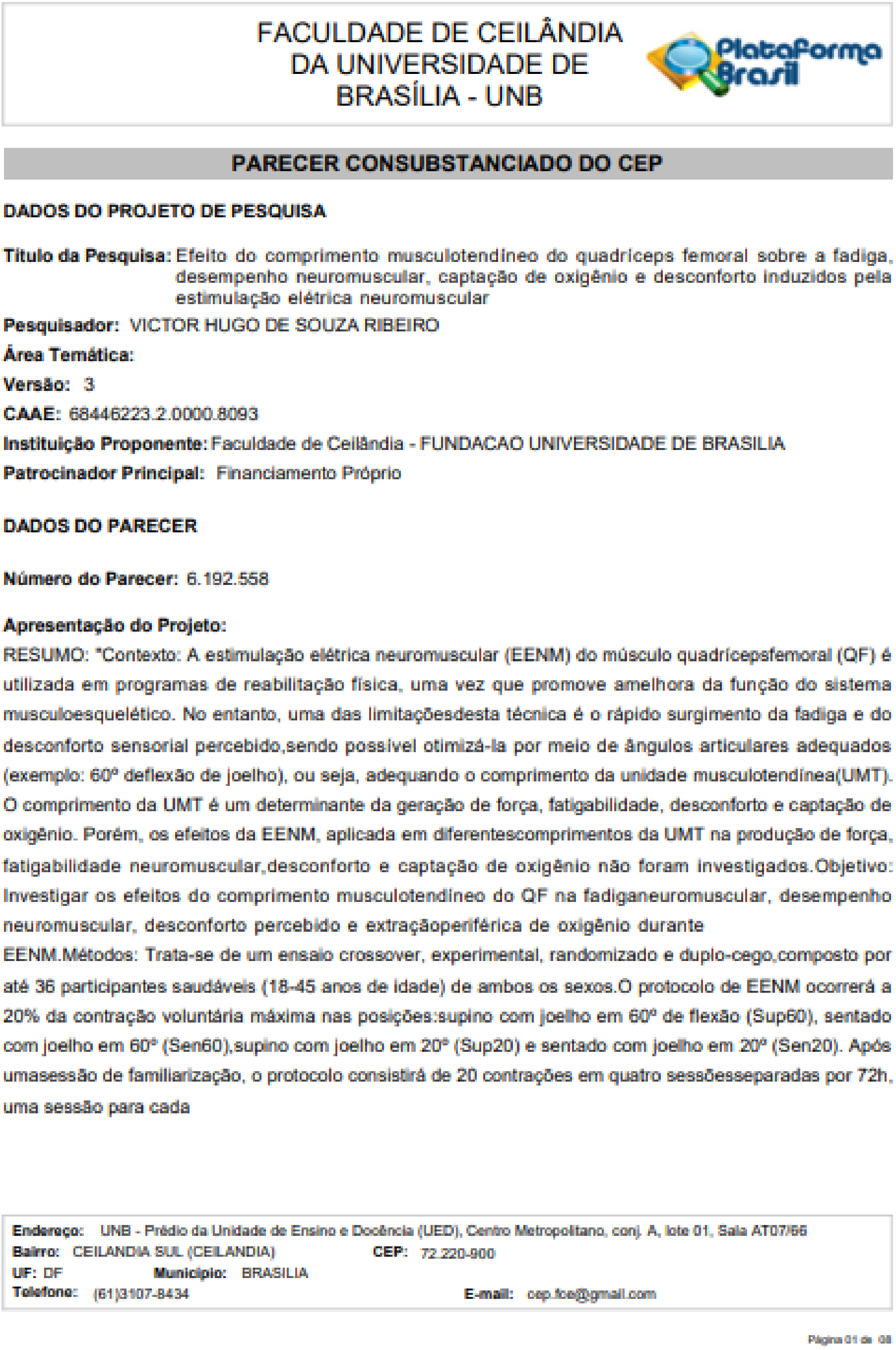
12. APPENDICES

12.1 Appendix I - Research ethics committee approval



FACULDADE DE CEILÂNDIA
DA UNIVERSIDADE DE
BRASÍLIA - UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito do comprimento musculotendíneo do quadríceps femoral sobre a fadiga, desempenho neuromuscular, captação de oxigênio e desconforto induzidos pela estimulação elétrica neuromuscular

Pesquisador: VICTOR HUGO DE SOUZA RIBEIRO

Área Temática:

Versão: 3

CAAE: 68446223.2.0000.8093

Instituição Proponente: Faculdade de Ceilândia - FUNDACAO UNIVERSIDADE DE BRASILIA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.192.558

Apresentação do Projeto:

RESUMO: "Contexto: A estimulação elétrica neuromuscular (EENM) do músculo quadrícepsfemoral (QF) é utilizada em programas de reabilitação física, uma vez que promove amelhora da função do sistema musculoesquelético. No entanto, uma das limitaçõesdesta técnica é o rápido surgimento da fadiga e do desconforto sensorial percebido,sendo possível otimizá-la por meio de ângulos articulares adequados (exemplo: 60º deflexão de joelho), ou seja, adequando o comprimento da unidade musculotendínea(UMT). O comprimento da UMT é um determinante da geração de força, fatigabilidade, desconforto e captação de oxigênio. Porém, os efeitos da EENM, aplicada em diferentescomprimentos da UMT na produção de força, fatigabilidade neuromuscular,desconforto e captação de oxigênio não foram investigados.Objetivo: Investigar os efeitos do comprimento musculotendíneo do QF na fadiganeuromuscular, desempenho neuromuscular, desconforto percebido e extraçãoperiférica de oxigênio durante
EENM.Métodos: Trata-se de um ensaio crossover, experimental, randomizado e duplo-cego,composto por até 36 participantes saudáveis (18-45 anos de idade) de ambos os sexos.O protocolo de EENM ocorrerá a 20% da contração voluntária máxima nas posições:supino com joelho em 60º de flexão (Sup60), sentado com joelho em 60º (Sen60),supino com joelho em 20º (Sup20) e sentado com joelho em 20º (Sen20). Após umasessão de familiarização, o protocolo consistirá de 20 contrações em quatro sessõesseparadas por 72h, uma sessão para cada

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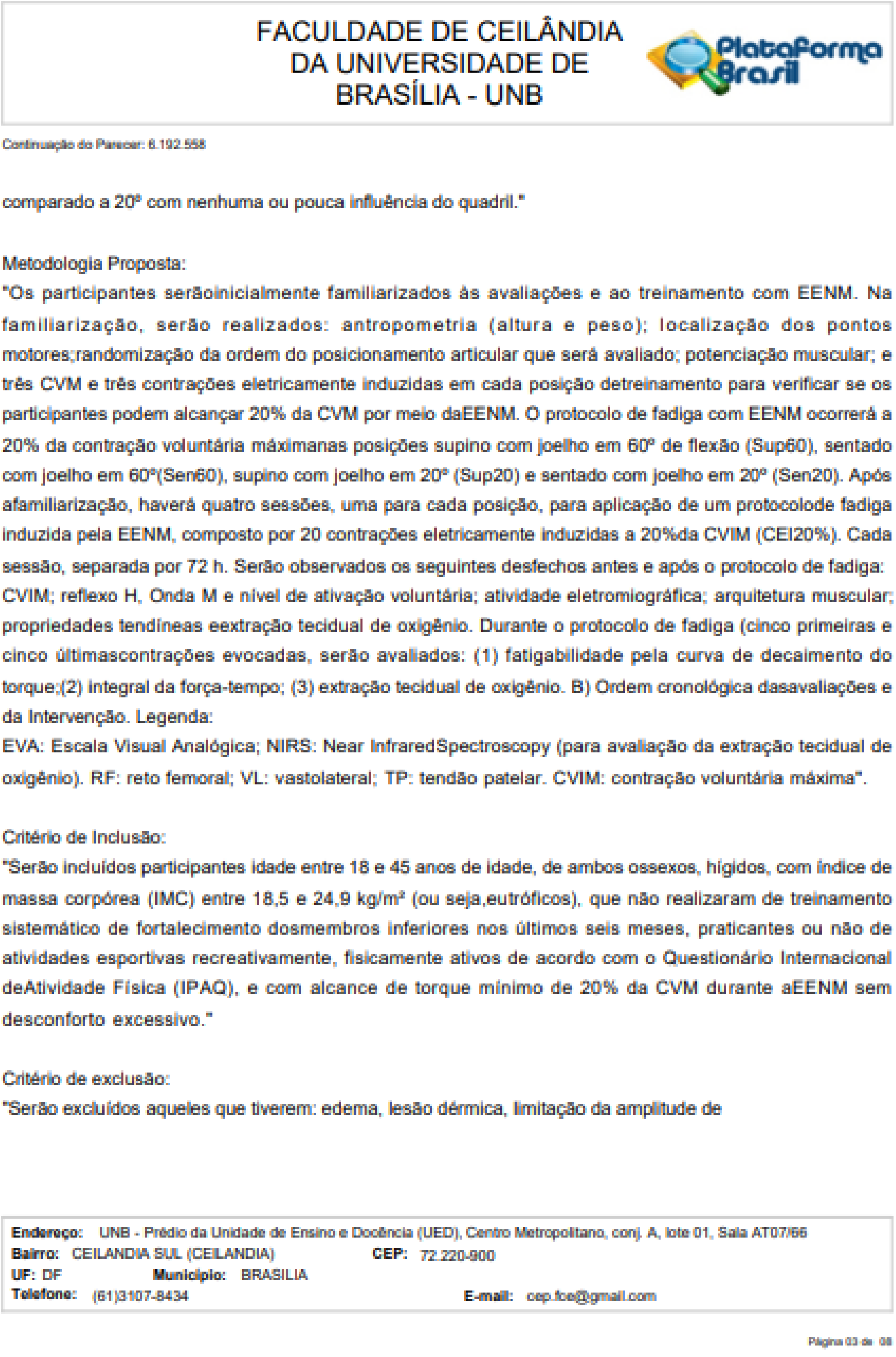
posicionamento. Os desfechos antes e após o protocolo de fadiga serão a contração voluntária, o nível de ativação voluntária, a ondaM, o reflexo H, a ativação muscular (Root Mean Square), a frequência de ativação, aarquitetura muscular, as propriedades tendíneas, o desconforto sensorial e a extraçãotecidual de oxigênio. Além disso, a variação da arquitetura muscular, as propriedadestendíneas e o desconforto sensorial serão avaliados durante contrações voluntárias antesa após a fadiga induzida pela EENM.Resultados esperados: Espera-se que com o joelho a 60º, independentemente daposição do quadril, haja maior fatigabilidade, porém menor desconforto sensorial noQF. A fadiga será explicada por fatores centrais e periféricos, predominando menoreficiência da contratilidade e captação de oxigênio nas posições encurtadas e menorativação central nas posições alongadas."

Hipótese:

"A fadiga voluntária será maior com o joelho em 60º de flexão de joelho, comparada com20º, podendo ou não ser maior quando o quadril estiver estendido, comparado com fletido; O reflexo H, a onda M, o nível de ativação voluntária, a RMS e a frequência medianapoderão estar mais reduzidos com o joelho a 20º após fadiga com EENM, devido fadigacentral em detrimento da periférica. O quadril estendido poderá reduzir a fadiga central eaumentar a fadiga periférica; A fadiga evocada (variação do torque evocado iniciando a 20% da CVIM e do tempointegral de torque) será maior com o joelho em 60º de flexão de joelho, comparada com20º, podendo ou não ser maior quando o quadril estiver estendido, comparado com fletido; A variação do p e do Cf durante a MVIC reduzirão proporcionalmente ao torque se nãohouver o fator confundidor da redução da rigidez das estruturas tendíneas, conformehipótese abaixo; A espessura muscular, cuja variação é mais relevante no repouso,indicará a presença de edema muscular, que será maior nas posições com o joelho a 60º; A rigidez do CTA poderá ser reduzida após o protocolo de fadiga para as posições maisalongadas; As propriedades tendíneas poderão estar alteradas (menor rigidez e menor móduloelástico) reduzida após o protocolo de fadiga para as posições mais alongadas; A integral de força-tempo será em média maior nas posições com o joelho a 60º, porém,esta reduzirá mais ao longo da sessão, indicando maior fadiga com o joelho a 60º; A extração tecidual de oxigênio será maior quando maior torque for desenvolvido,independente da atividade eletromiográfica; Independentemente da posição do quadril, menor amplitude de corrente será requerida com o joelho a 60º para manter o torque a 20% da CVM. Portanto, haverá maior eficiênciada corrente em 60º comparado a 20º; Será reportada menor percepção de desconforto com o joelho a 60º

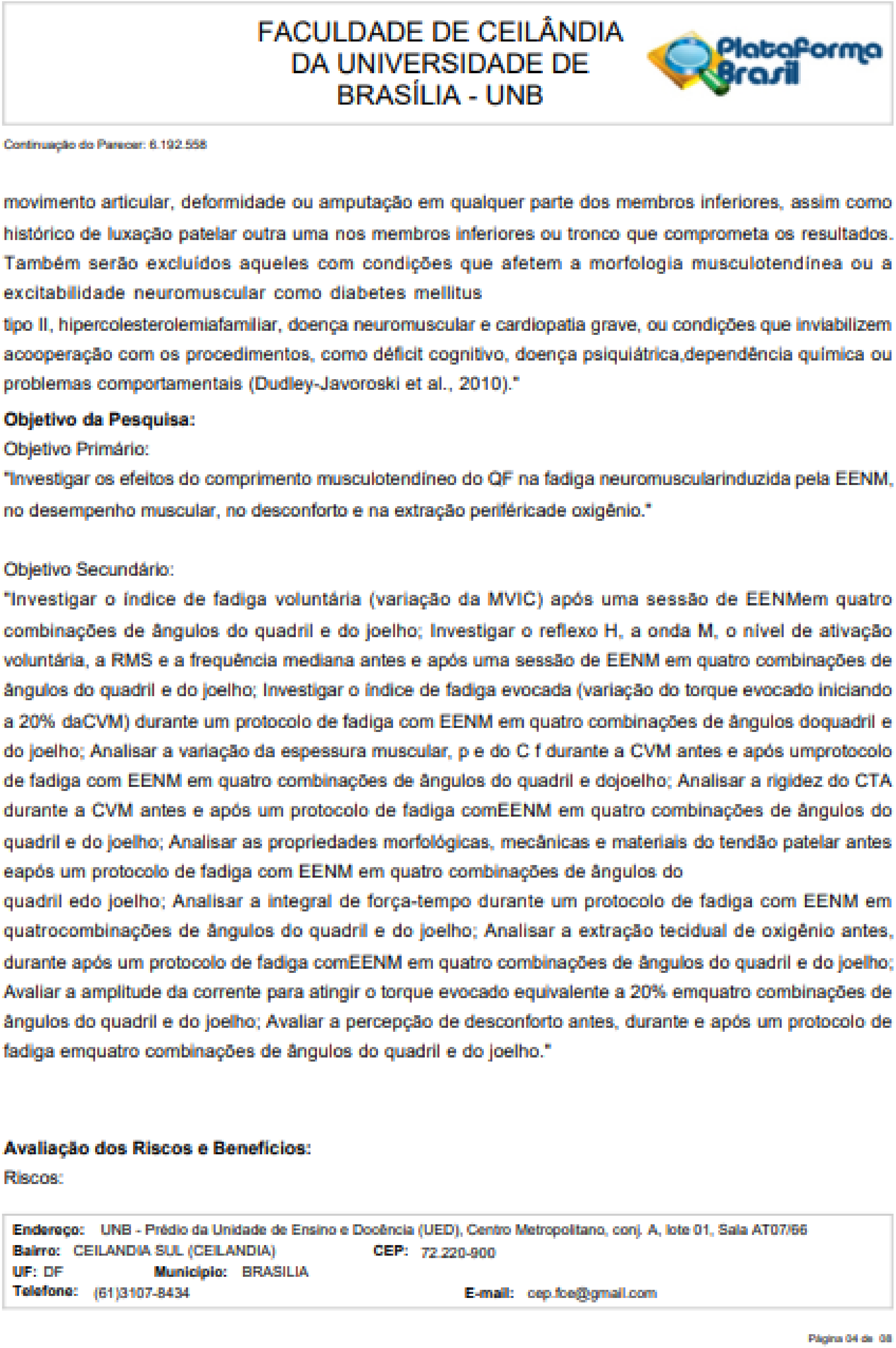


comparado a 20º com nenhuma ou pouca influência do quadril."

Metodologia Proposta:
"Os participantes serãoinicialmente familiarizados às avaliações e ao treinamento com EENM. Na familiarização, serão realizados: antropometria (altura e peso); localização dos pontos motores;randomização da ordem do posicionamento articular que será avaliado; potenciação muscular; e três CVM e três contrações eletricamente induzidas em cada posição detreinamento para verificar se os participantes podem alcançar 20% da CVM por meio daEENM. O protocolo de fadiga com EENM ocorrerá a 20% da contração voluntária máximanas posições supino com joelho em 60º de flexão (Sup60), sentado com joelho em 60º(Sen60), supino com joelho em 20º (Sup20) e sentado com joelho em 20º (Sen20). Após afamiliarização, haverá quatro sessões, uma para cada posição, para aplicação de um protocolode fadiga induzida pela EENM, composto por 20 contrações eletricamente induzidas a 20%da CVIM (CEI20%). Cada sessão, separada por 72 h. Serão observados os seguintes desfechos antes e após o protocolo de fadiga:
CVIM; reflexo H, Onda M e nível de ativação voluntária; atividade eletromiográfica; arquitetura muscular; propriedades tendíneas eextração tecidual de oxigênio. Durante o protocolo de fadiga (cinco primeiras e cinco últimascontrações evocadas, serão avaliados: (1) fatigabilidade pela curva de decaimento do torque;(2) integral da força-tempo; (3) extração tecidual de oxigênio. B) Ordem cronológica dasavaliações e da Intervenção. Legenda:
EVA: Escala Visual Analógica; NIRS: Near InfraredSpectroscopy (para avaliação da extração tecidual de oxigênio). RF: reto femoral; VL: vastolateral; TP: tendão patelar. CVIM: contração voluntária máxima".

Critério de Inclusão:
"Serão incluídos participantes idade entre 18 e 45 anos de idade, de ambos ossexos, hígidos, com índice de massa corpórea (IMC) entre 18,5 e 24,9 kg/m^2 (ou seja,eutróficos), que não realizaram de treinamento sistemático de fortalecimento dosmembros inferiores nos últimos seis meses, praticantes ou não de atividades esportivas recreativamente, fisicamente ativos de acordo com o Questionário Internacional deAtividade Física (IPAQ), e com alcance de torque mínimo de 20% da CVM durante aEENM sem desconforto excessivo."

Critério de exclusão:
"Serão excluídos aqueles que tiverem: edema, lesão dérmica, limitação da amplitude de



movimento articular, deformidade ou amputação em qualquer parte dos membros inferiores, assim como histórico de luxação patelar outra uma nos membros inferiores ou tronco que comprometa os resultados. Também serão excluídos aqueles com condições que afetem a morfologia musculotendínea ou a excitabilidade neuromuscular como diabetes mellitus
tipo II, hipercolesterolemiafamiliar, doença neuromuscular e cardiopatia grave, ou condições que inviabilizem acooperação com os procedimentos, como déficit cognitivo, doença psiquiátrica,dependência química ou problemas comportamentais (Dudley-Javoroski et al., 2010)."

Objetivo da Pesquisa:

Objetivo Primário:

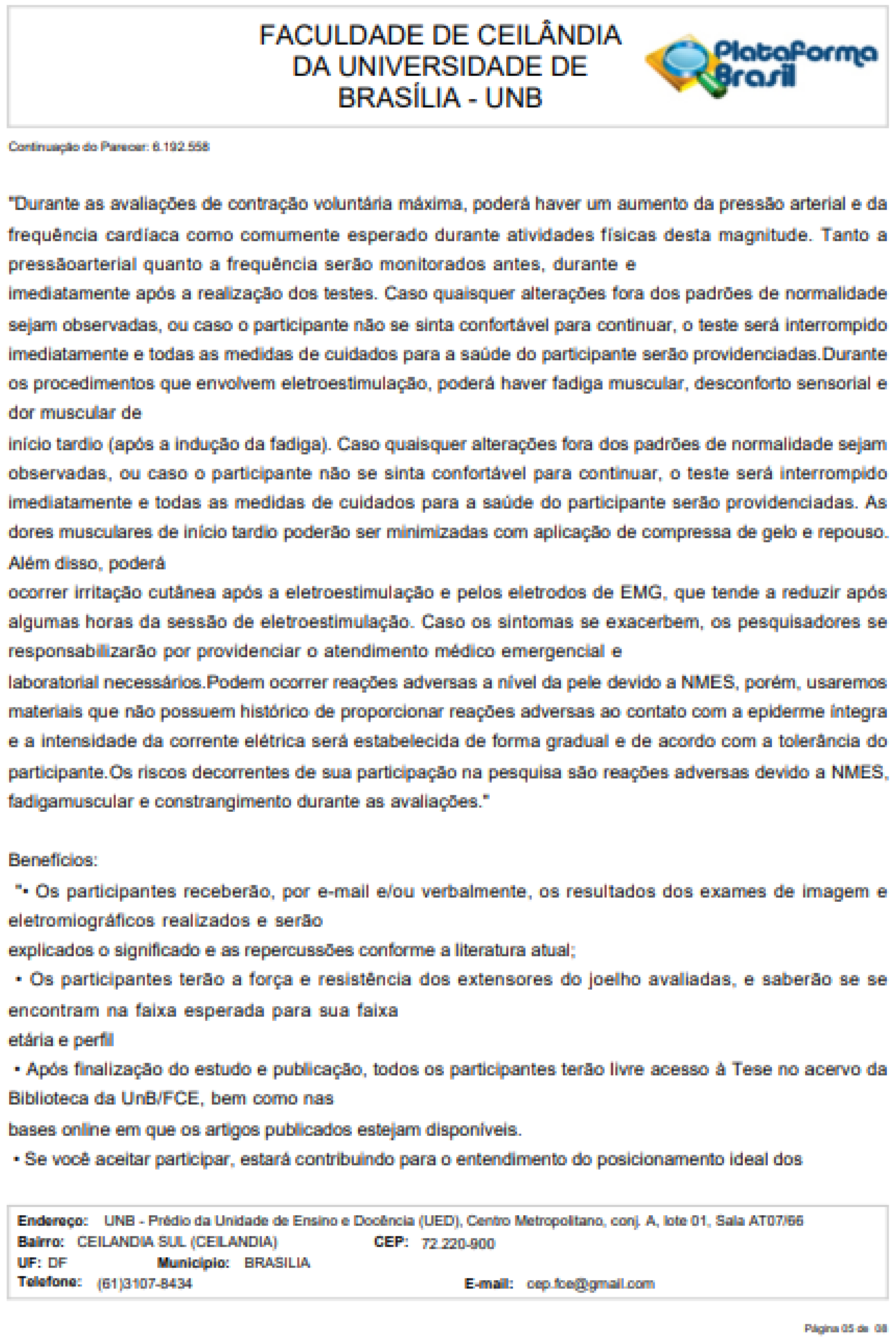
"Investigar os efeitos do comprimento musculotendíneo do QF na fadiga neuromuscularinduzida pela EENM, no desempenho muscular, no desconforto e na extração periféricade oxigênio."

Objetivo Secundário:

"Investigar o índice de fadiga voluntária (variação da MVIC) após uma sessão de EENMem quatro combinações de ângulos do quadril e do joelho; Investigar o reflexo H, a onda M, o nível de ativação voluntária, a RMS e a frequência mediana antes e após uma sessão de EENM em quatro combinações de ângulos do quadril e do joelho; Investigar o índice de fadiga evocada (variação do torque evocado iniciando a 20% daCVM) durante um protocolo de fadiga com EENM em quatro combinações de ângulos doquadril e do joelho; Analisar a variação da espessura muscular, p e do C f durante a CVM antes e após umprotocolo de fadiga com EENM em quatro combinações de ângulos do quadril e dojoelho; Analisar a rigidez do CTA durante a CVM antes e após um protocolo de fadiga comEENM em quatro combinações de ângulos do quadril e do joelho; Analisar as propriedades morfológicas, mecânicas e materiais do tendão patelar antes eapós um protocolo de fadiga com EENM em quatro combinações de ângulos do
quadril edo joelho; Analisar a integral de força-tempo durante um protocolo de fadiga com EENM em quatrocombinações de ângulos do quadril e do joelho; Analisar a extração tecidual de oxigênio antes, durante após um protocolo de fadiga comEENM em quatro combinações de ângulos do quadril e do joelho; Avaliar a amplitude da corrente para atingir o torque evocado equivalente a 20% emquatro combinações de ângulos do quadril e do joelho; Avaliar a percepção de desconforto antes, durante e após um protocolo de fadiga emquatro combinações de ângulos do quadril e do joelho."

Avaliação dos Riscos e Benefícios:

Riscos:



"Durante as avaliações de contração voluntária máxima, poderá haver um aumento da pressão arterial e da frequência cardíaca como comumente esperado durante atividades físicas desta magnitude. Tanto a pressãoarterial quanto a frequência serão monitorados antes, durante e imediatamente após a realização dos testes. Caso quaisquer alterações fora dos padrões de normalidade sejam observadas, ou caso o participante não se sinta confortável para continuar, o teste será interrompido imediatamente e todas as medidas de cuidados para a saúde do participante serão providenciadas.Durante os procedimentos que envolvem eletroestimulação, poderá haver fadiga muscular, desconforto sensorial e dor muscular de início tardio (após a indução da fadiga). Caso quaisquer alterações fora dos padrões de normalidade sejam observadas, ou caso o participante não se sinta confortável para continuar, o teste será interrompido imediatamente e todas as medidas de cuidados para a saúde do participante serão providenciadas. As dores musculares de início tardio poderão ser minimizadas com aplicação de compressa de gelo e repouso. Além disso, poderá ocorrer irritação cutânea após a eletroestimulação e pelos eletrodos de EMG, que tende a reduzir após algumas horas da sessão de eletroestimulação. Caso os sintomas se exacerbem, os pesquisadores se responsabilizarão por providenciar o atendimento médico emergencial e laboratorial necessários.Podem ocorrer reações adversas a nível da pele devido a NMES, porém, usaremos materiais que não possuem histórico de proporcionar reações adversas ao contato com a epiderme íntegra e a intensidade da corrente elétrica será estabelecida de forma gradual e de acordo com a tolerância do participante.Os riscos decorrentes de sua participação na pesquisa são reações adversas devido a NMES, fadigamuscular e constrangimento durante as avaliações."

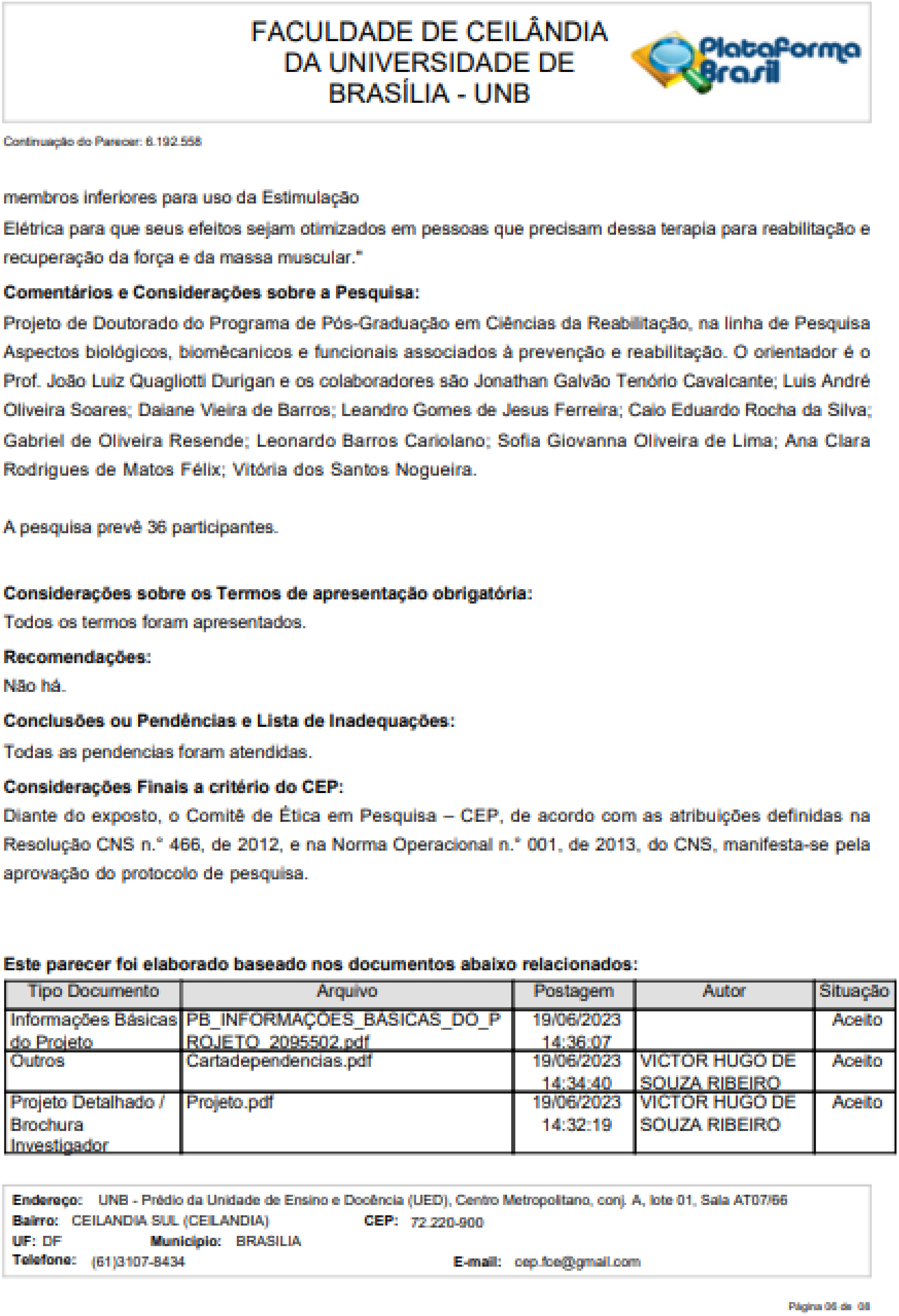
Benefícios:

"• Os participantes receberão, por e-mail e/ou verbalmente, os resultados dos exames de imagem e eletromiográficos realizados e serão explicados o significado e as repercussões conforme a literatura atual;

• Os participantes terão a força e resistência dos extensores do joelho avaliadas, e saberão se se encontram na faixa esperada para sua faixa etária e perfil

• Após finalização do estudo e publicação, todos os participantes terão livre acesso à Tese no acervo da Biblioteca da UnB/FCE, bem como nas bases online em que os artigos publicados estejam disponíveis.

• Se você aceitar participar, estará contribuindo para o entendimento do posicionamento ideal dos



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membros inferiores para uso da Estimulação

Elétrica para que seus efeitos sejam otimizados em pessoas que precisam dessa terapia para reabilitação e recuperação da força e da massa muscular."

Comentários e Considerações sobre a Pesquisa:

Projeto de Doutorado do Programa de Pós-Graduação em Ciências da Reabilitação, na linha de Pesquisa Aspectos biológicos, biomêcanicos e funcionais associados à prevenção e reabilitação. O orientador é o Prof. João Luiz Quagliotti Durigan e os colaboradores são Jonathan Galvão Tenório Cavalcante; Luis André Oliveira Soares; Daiane Vieira de Barros; Leandro Gomes de Jesus Ferreira; Caio Eduardo Rocha da Silva; Gabriel de Oliveira Resende; Leonardo Barros Cariolano; Sofia Giovanna Oliveira de Lima; Ana Clara Rodrigues de Matos Félix; Vitória dos Santos Nogueira.

A pesquisa prevê 36 participantes.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram apresentados.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

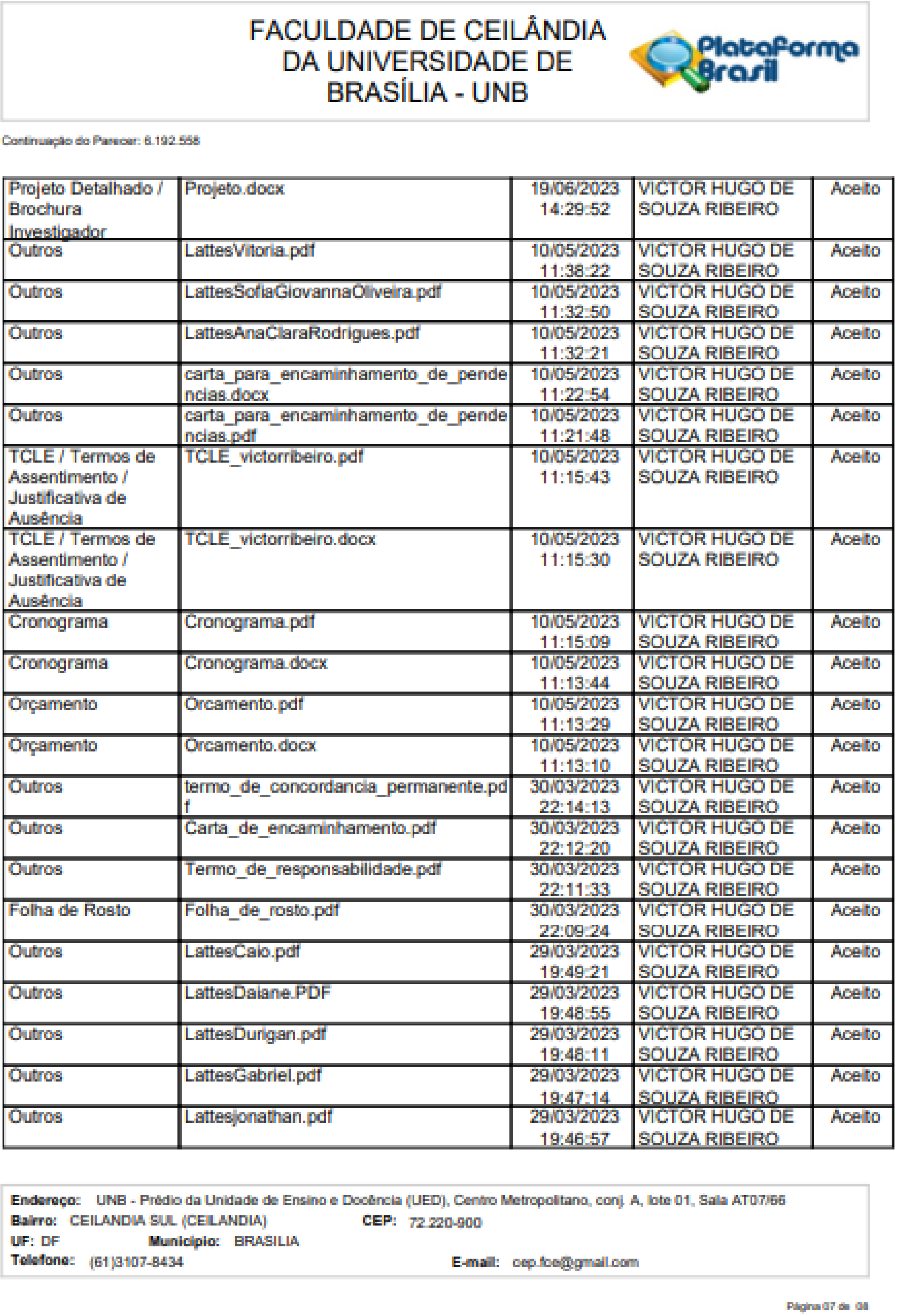
Todas as pendencias foram atendidas.

Considerações Finais a critério do CEP:

Diante do exposto, o Comitê de Ética em Pesquisa – CEP, de acordo com as atribuições definidas na Resolução CNS n.º 466, de 2012, e na Norma Operacional n.º 001, de 2013, do CNS, manifesta-se pela aprovação do protocolo de pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2095502.pdf	19/06/2023 14:36:07		Aceito
Outros	Cartadependencias.pdf	19/06/2023 14:34:40	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	19/06/2023 14:32:19	VICTOR HUGO DE SOUZA RIBEIRO	Aceito



Continuação do Parecer: 6.192.558

Projeto Detalhado / Brochura Investigador	Projeto.docx	19/06/2023 14:29:52	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
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Outros	LattesSofiaGiovannaOliveira.pdf	10/05/2023 11:32:50	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
Outros	LattesAnaClaraRodrigues.pdf	10/05/2023 11:32:21	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
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Outros	carta_para_encaminhamento_de_pendencias.pdf	10/05/2023 11:21:48	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_victorribeiro.pdf	10/05/2023 11:15:43	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_victorribeiro.docx	10/05/2023 11:15:30	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
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Orçamento	Orcamento.docx	10/05/2023 11:13:10	VICTOR HUGO DE SOUZA RIBEIRO	Aceito
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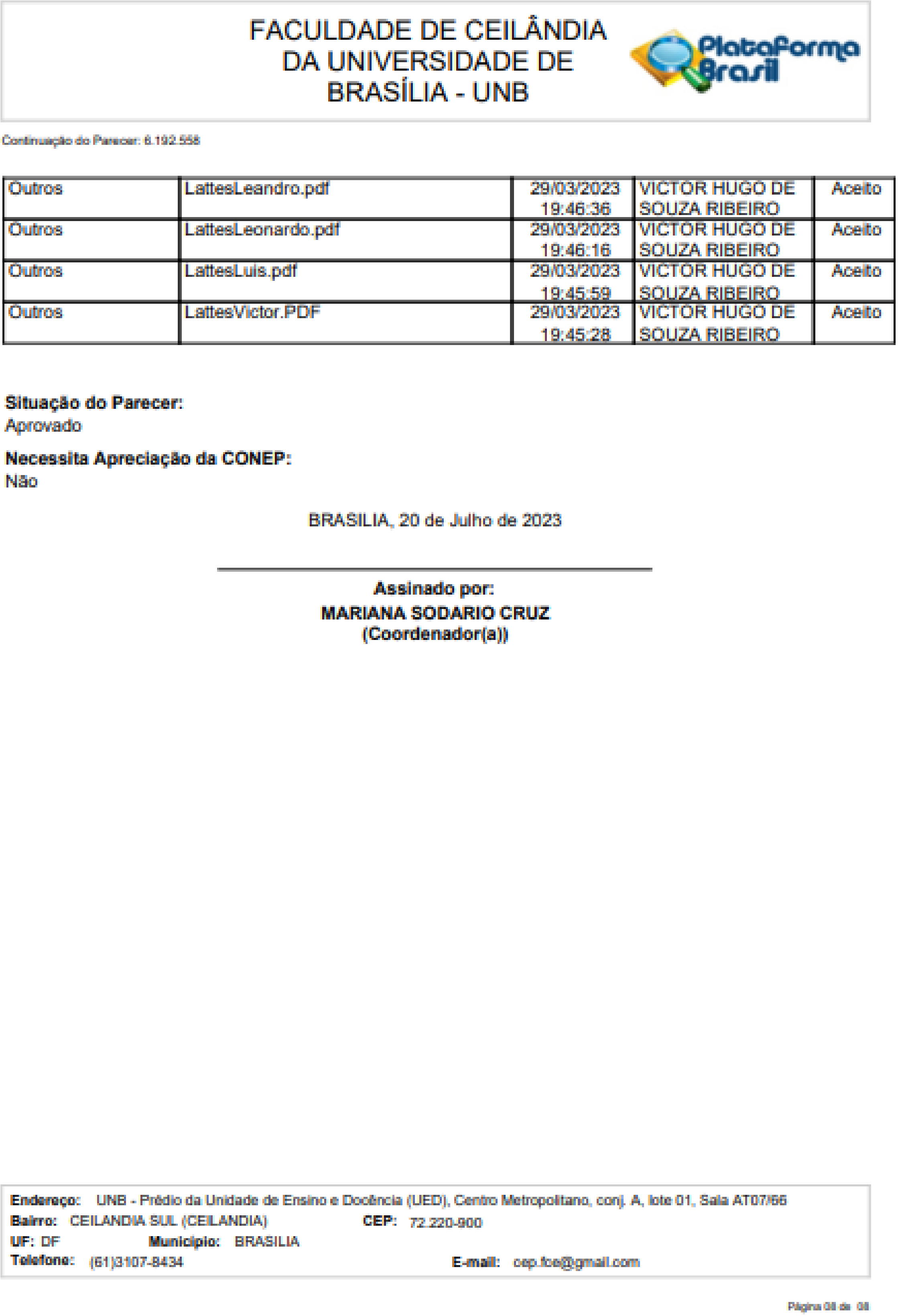

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Outros	LattesVictor.PDF	29/03/2023 19:45:28	VICTOR HUGO DE SOUZA RIBEIRO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRASILIA, 20 de Julho de 2023

Assinado por:
MARIANA SODARIO CRUZ
(Coordenador(a))

12.2 Appendix II – Suplementary table 1

Supplementary Table 1. Type III Analysis of Variance Table of KPa.

	Sum SQ	**Mean square**	**Df**	**F value**	**P value**
Positions	1948.23	649	3	74.138	< 0.001
Muscles	112.23	112.23	1	12.48	0.001
Depht	538.74	269.37	2	35.54	< 0.001
Positions × Muscles	261.31	87.10	3	14.15	< 0.001
Positions × Depht	33.04	5.51	6	3.39	0.003
Muscles × Depht	4.26	2.13	2	1.81	0.171
Positions × Muscles × Depht	1.06	0.18	6	0.16	0.98

Sum SQ: Sum of squares

Df: degrees of freedom

12.3 Appendix III – Suplementary table 2

Supplementary Table 2. Type III Analysis of Variance Table of m/S.

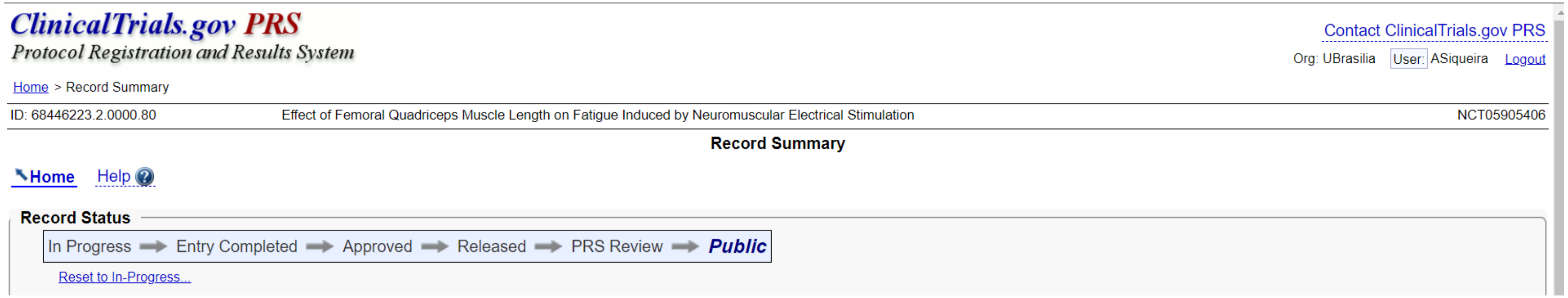
	Sum SQ	**Mean square**	**Df**	**F value**	**P value**
Positions	16.56	5.52	3	63.93	< 0.001
Muscles	0.96	0.96	1	9.71	0.004
Depht	4.32	2.16	2	30.19	< 0.001
Positions × Muscles	1.55	0.51	3	9.13	< 0.001
Positions × Depht	0.30	0.05	6	3.10	0.006
Muscles × Depht	0.05	0.02	2	1.99	0.145
Positions × Muscles × Depht	0.01	0.00	6	0.16	0.98

Sum SQ: Sum of squares

Df: degrees of freedom

DenDF: DF in the denominator

12.4 Appendix IV - Research ethics committee approval



ClinicalTrials.gov PRS
Protocol Registration and Results System

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Home > Record Summary

ID: 68446223.2.0000.80 Effect of Femoral Quadriceps Muscle Length on Fatigue Induced by Neuromuscular Electrical Stimulation NCT05905406

Record Summary

Record Status

In Progress ➡ Entry Completed ➡ Approved ➡ Released ➡ PRS Review ➡ ***Public***