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Neuroelectrostimulation on Adult Mice's Back and Hip Muscles, Through a Special Vest: a Pilot Study

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ABSTRACT

Introduction: neuromuscular electrostimulation (NMES) is a promising strategy for recovering muscle mass and function, including in elderly patients suffering from sarcopenia. We developed a vest with coupled electrodes to introduce electrostimulation throughout the body of young mice and verify the morphological changes in the scapular and pelvic region muscles.

Material and Methods: a vest based on the semi-flexible resin was developed with the electrodes already attached, printed by 3D printing with connectivity to the Neurodyn III electrostimulation device (Ibramed ®). Based on this, six 14-weeks old C57BL/6 WT mice were used to conduct this pilot study.

Results: After four weeks, we found that using the vest with the coupled electrodes was able to generate an increase in CSA in the gluteus and trapezius muscle compared to control mice ($p<0.05$). The gluteus of the experimental group (EG) hypertrophied 15% more than the control group (CG). As occurred in the gluteus, in the trapezius muscle, the CSA was 37% higher in EG than in CG.

Conclusions: we conclude that this model of NMES in mice leaded to morphological changes in scapular and pelvic region muscles. For future work, we intend to include molecular and functional analyzes to evaluate if our model can reproduce the same functional effects reported in studies and afterward apply this model to elderly animals.

Keywords: Electrostimulation; Skeletal Muscle; Hypertrophy; Sarcopenia.

Introduction

The knowledge of electrophysiology is dated from the beginning of the 18th century and, over time, after several experiments based on observations and empirical knowledge, it was found that the nature of transmission of the nerves impulses was directly related to an electrical current propagating through the nerve[1]. It is transmitted from the nerve to the muscle through the motor units, transforming the electrical signal into contractile activity[2].

In this context, the force exerted by the muscle during voluntary contractions depends on the number of motor units activated and the speed that they can discharge into their action potentials[3]. Motor units undergo rearrangements over the years, for instance, in individuals with advanced age or stricken with neurological diseases, the motor unit activation may be compromised[2].

Therefore, neuromuscular electrostimulation (NMES) is a tool for recovering from injuries that affected muscle tissue. Besides, its use has been described as an aid to recovering muscle mass and function, including in elderly patients suffering from sarcopenia, as an alternative treatment[4,5,6].

Sarcopenia is defined as a progressive and generalized disorder of skeletal muscle, which involves accelerated loss of muscle mass and function[7]. However, the parameter "muscle function," also described as "muscle strength," has shown greater clinical relevance than isolated muscle mass[8])a great deal of research has focused on identifying strategies to maintain muscle mass during the aging process and elucidating key molecular pathways of atrophy, with the rationale that the loss of strength is primarily a direct result of the age-associated declines in mass (sarcopenia, as already mentioned in the literature, that electrostimulation can provide improvement in muscle strength of elderly people with percentages ranging from 50 to 80%[9].

Thus, the results that show improvement in muscle strength after electrostimulation is related to greater stimulation in type II fibers and increased protein synthesis after the sessions [(10)]. In addition, another study reports that, after a high frequency (60Hz)

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electrostimulation session in the vastus lateralis muscle, mTOR increased by 68%[11].

Recently, a new electrical stimulation protocol has shown promising results and satisfactory muscle function parameters such as strength, power, and hypertrophy [12]. This new approach stimulates the muscles of the whole body, increasing the efficiency of training in a shorter period[13].

Hence, the objective of this pilot study was to develop a vest with coupled electrodes to introduce electrostimulation throughout the body of young mice and verify the morphological changes in the muscles of the scapular and pelvic region. Therefore, applying this methodology to elderly animals may be demonstrated morphological parameters and muscle function. To date, no studies have been found in the literature that has used electrostimulation in the entire body of mice.

Materials and Methods

Animals

Six 14-weeks old C57BL/6 WT mice were used, which were provided from São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. The animals were housed in conventional cages containing three animals each by the Animal Laboratory of Araçatuba School of Dentistry – São Paulo State University (UNESP), with feeders and drinkers "ad libitium" (irradiated feed – Nuvilab rodents and filtered water) at temperature-controlled rooms (22-25ºC). All experiments procedures in the animals were conducted with the approval of the Institutional Ethics Committee on Animal Use of Araçatuba School of Dentistry, São Paulo State University (UNESP) protocol #0270-2021.

Experimental design

The animals were divided into two groups: Control Group (C.G) – Consisted of three animals that did not receive electrical stimulation and Experimental Group (E.G) – Consisted of three animals that received electrical stimulation. Based on this, to conduct the electrical impulses, a vest based on the semi-flexible resin was developed with the electrodes already attached (Figure 1), printed by 3D printing with connectivity to the Neurodyn III electrostimulation device (Ibramed®), used to carry out this study. Before the experiment, the animals were previously anesthetized by intramuscular injection with 100mg/kg ketamine chloride (Dopalen, Agribrands Brasil, Paulínia, S.P., Brazil) and 5mg/kg xylazine chloride (Anasedan, Agribrands Brasil, Paulínia, S.P., Brazil). After the animals were anesthetized, they were dressed in vests for the beginning of the experiment, which lasted four weeks. A rectangular symmetrical biphasic pulsed current was used to conduct the electrostimulation with a frequency of 80 Hz, 400 microseconds of pulse duration, 15 seconds ON and 30 seconds OFF, in the proportion of 1:2 described by (DOUCET; LAM; GRIFFIN, 2012)[14], lasting 25 minutes, 1x per week. From the 3rd week of the experiment, we increased the time to 30 minutes, raising the session volume, similarly as in resistance training and the intensity, which went from 2mA to 3mA, until the last session, before sacrifice.

At the end of the experiment, the animals were euthanatized according to the protocols approved by the Guidelines for the Care and Use of Laboratory Animals. We dissected the gluteus and scapular muscles, which were immediately frozen in liquid nitrogen and stored at -80ºC for later histological analysis.

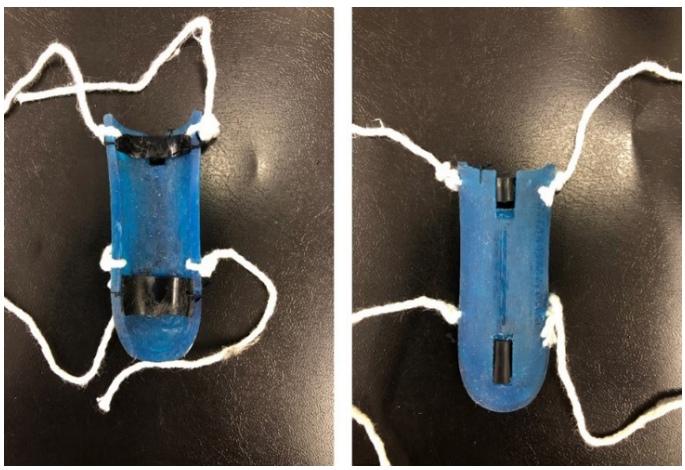

Figure 1. Special vest, printed out by a 3D printer and elaborated by the authors.

Histological Processing of Frozen Muscles

After the mice were euthanized, the gluteus and trapezius muscles were removed and embedded into the OCT (Optimal Cutting Temperature) (Tissue-Tek; Sakura Finetek, Torrance, CA, USA) and frozen in liquid nitrogen and stored at -80ºC. We have positioned the electrodes aiming to target a wide range of muscles, stimulating through the brachial plexus and sciatic nerve. Nevertheless, we choose the muscles due to the kinesiological and biomechanical functions that they exercise in animals. The gluteus muscle is the primary pelvic stabilizer. Studies have shown that functional damage occurs in the hip joint in cases of muscle weakness and a vast degradation of bone density and strength in the greater trochanter of the femur[15]. Thus, contributing to the development of osteoporosis in animals.

On the other hand, the trapezius muscle controls the alignment of the scapula and its movements[16]. Besides, it participates in most of the actions of the upper limbs, being also a stability muscle[16]. All the samples were brought to -22 ºC, and serial transverse sections (8µm) were made with a cryostat (Leica 1850).

Hence, histological slides were stained with hematoxylin and eosin (H&E) to measure the muscle fiber's cross-sectional area (CSA) (Figure 2). The microscope (model Olympus BX50) with 40x magnification was used to capture the images at the anatomy department of Bauru School of Dentistry, University of São Paulo (FOB-USP). We used the SigmaProScan 5.0 software, bypassing the perimeter of each muscle fiber individually from each muscle, from each animal by the group to evaluate the muscle fiber's CSA. We measured 100 muscle fiber's from each sample from each animal per group.

Statistical Analysis

Quantitative data were first analyzed for distribution of normality using the Shapiro-Wilk normality test. We used the t-test to evaluate the effect of electrostimulation for quantitative parameters to identify differences in the mean values for the C.G and E.G

Results

The perimeter of the muscle fibers of the gluteus and trapezius muscles was traced to obtain the cross-sectional area (CSA). In both muscles, the group stimulated with electrostimulation – Experimental Group (E.G) showed a greater CSA with statistically significant difference in comparison with non-stimulated mice (Figure 3). As a result, we found that using the vest with the coupled electrodes, developed to carry out this pilot study for the application of electrostimulation, was able to generate an increase in CSA after four weeks of the experiment.

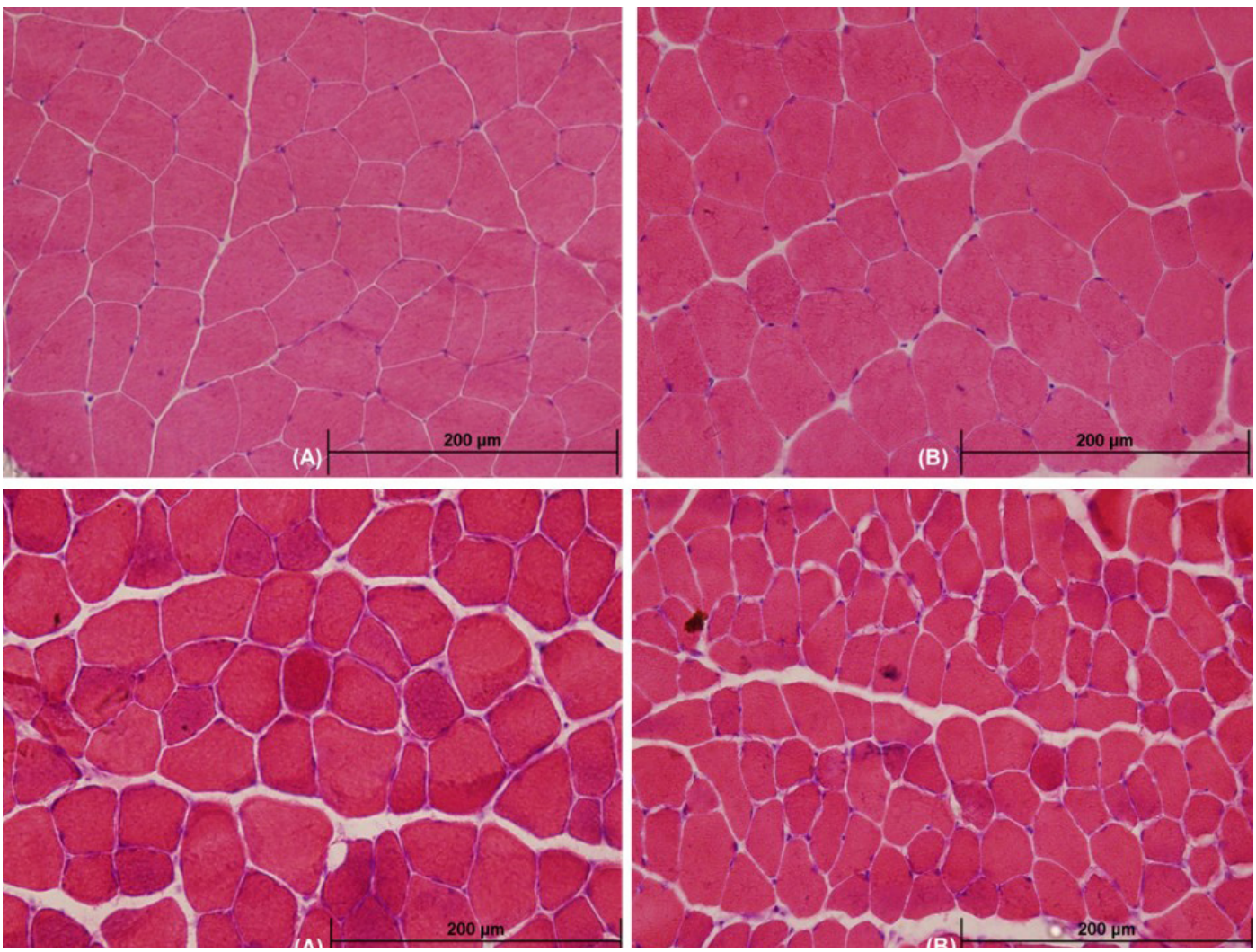

Figure 2 . Hematoxylin and eosin staining on gluteus and trapezius muscles for trace and obtain the CSA – (A) CG gluteus muscle; (B) EG gluteus muscle; (A) EG trapezius muscle; (B) CG trapezius muscle.

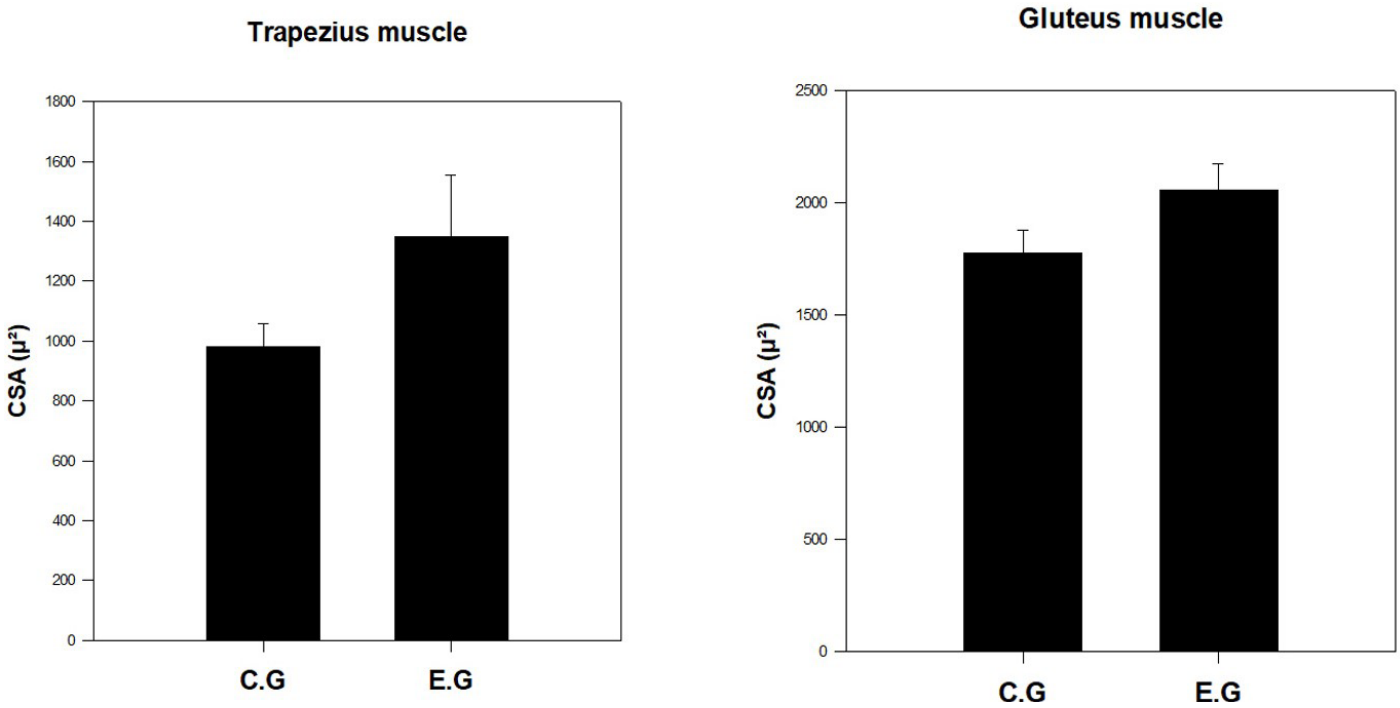

Figure 3. The mean cross-sectional area (µ²) of gluteus and trapezius muscles. Values are means + S.D. Significant differences between groups: P< 0,05.

Discussion

In our pilot study, we aimed to develop and test a vest with electrodes attached and adapted for electrostimulation in the whole body of young mice to verify the morphological changes in the muscles of the scapular and pelvic region. It is supported by the literature since, both in animals and humans, consistent data have been showing that electrostimulation is capable of generating changes in skeletal muscle[17,18,19]. In agreement with other researchers, the results contemplated in this study showed significant differences between the cross-sectional areas (CSA) of the gluteus and trapezius muscles between the control and experimental group after four weeks of stimulation.

The mechanism associated with changes in muscle fibers through electrostimulation is similar to that of resistance training since it significantly increases the mTOR signaling, a key marker in protein synthesis cascades[20]. Furthermore, studies using high-frequency electrostimulation, such as 60Hz, showed an increase of mTOR up to 68%[11]. Moreover, a frequency of 80Hz, also considered high, was used since other studies have observed significant increases in more than one protein from the synthesis cascades[21].

In addition to the morphological changes of the muscle, electrostimulation is described as a training method capable of increasing muscle strength, with studies reporting increases of 10 to 41% in the quadriceps muscles[22,23]. Therefore, this increase in strength may occur because electrostimulation preferentially stimulates type II fibers[10]. Likewise, the most vulnerable group affected by the aging process and falls are the elderly patients with a lack of muscle strength[24].

A new method of using electrostimulation has been applied to produce more significant results in a shorter period [13]. This new approach consists of applying electrostimulation throughout the whole body and generating substantial improvements in strength and hypertrophy [12]. In our study, we used a similar model that caused a significant difference in the CSA between the groups in agreement with other studies (Figure 3)[17,25].

Thus, we verified the viability of our model in generating hypertrophy after a 4-week trial period. For future work, we intend to include molecular and functional analyzes to evaluate if our model can reproduce the same functional effects reported in studies with humans using electrostimulation in the whole-body[12,13], and afterward to analyze the impact of this model on elderly animals.

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Mini Curriculum and Author's Contribution

1. João Vitor Tadashi Cosin Shindo – MsC student. Contribution: conceived and designed the experiments also, wrote the paper. ORCID: 0000-0002-0230-5962.

2. José Eduardo Petit Rodokas – Master degree. Contribution: He designed the vest for us, provided the 3D printer also enlightened us about what resin was better to attend to our demand. ORCID: 0000-0003-2683-9982

3. Guilherme dos Santos Sousa – PhD student; Contribution: Enlightened us about what device was better to attend our research also made technology improvements in the device to increase the number of animals that could be stimulated per round. ORCID: 0000-0001-6174-2794.

4. Fabio Oliveira Maciel – PhD. Contribution: Fabio is a teacher at the Federal University of Amazonas (UFAM) and has great experience in the electrostimulation field. The experiment's design was supervised by his support.

5. Maira Cristina Rondina Couto – BSc. Contribution: Maira helped during the experiments also with her skilled assistance in histological staining.

6. Mariza Akemi Matsumoto – PhD. Contribution: All the resources used in this work were provided by teacher Mariza Akemi Matsumoto. ORCID: 0000-0001-5389-0105

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