DOI: http://dx.doi.org/10.1590/1413-785220212906237983

THE EFFECTS OF NEUROMUSCULAR ELECTRICAL STIMULATION IN ASSOCIATION WITH WHEY PROTEIN SUPPLEMENTATION AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

EFEITOS DA ESTIMULAÇÃO ELÉTRICA NEUROMUSCULAR ASSOCIADO À SUPLEMENTAÇÃO COM WHEY PROTEIN APÓS RECONSTRUÇÃO DO LIGAMENTO CRUZADO ANTERIOR

Gabriela Otília Mendonça[1], Maria Luiza Bianchini Severino[1], Kelle Moreira de Oliveira[1], Marcelo Lima de Oliveira[1], Giovane Galdino de Souza[1], Adriano Prado Simão[1], Daniel Ferreira Moreira Lobato[2], Ranielly Alves Andrade[3], Ricardo Zenun Franco[4], Marcelo Stegmann da Cruz[5], Leonardo César Carvalho[1]

1. Federal University of Alfenas, Institute of Motricity Science, Alfenas, MG, Brazil.
2. Federal University of Triângulo Mineiro, Institute of Health Sciences, Alfenas, MG, Brazil.
3. Universidade José do Rosário Vellano, Medical School, Alfenas, MG, Brazil.
4. Federal University of Alfenas, Medical School, Alfenas, MG, Brazil.
5. Institute of Orthopedics and Traumatology, Alfenas, MG, Brazil.

ABSTRACT

Objective: To analyze the effects of neuromuscular electrical stimulation of the femoral quadriceps associated or not with whey protein supplementation on the electromyographic activity and body mass distribution in volunteers undergoing anterior cruciate ligament reconstruction. Methods: 24 volunteers were randomly divided into three groups: basal control, whey protein in association with neuromuscular electrical stimulation, and neuromuscular electrical stimulation alone. Results: In the postoperative evaluation, during the mini squat, the basal group showed a decrease in the electromyographic activity of the vastus medialis (p = 0.005, eyes open; p = 0.003, eyes closed), vastus lateralis (p = 0.005, eyes open; p = 0.020; eyes closed) and rectus femoris (p = 0.075, eyes open; p = 0.074, eyes closed) and of body mass distribution in the injured limb (p < 0.001, eyes open; p < 0.001, eyes closed), and in the healthy limb (p < 0.001, eyes open; p < 0.001, eyes closed). Conclusion: The early use of neuromuscular electrical stimulation of the quadriceps femoris maintained the electromyographic activity of the vastus medialis and vastus lateralis muscles and prevented asymmetries in body mass distribution 15 days after anterior cruciate ligament reconstruction. ***Level of Evidence I, High quality randomized trial.***

Keywords: Anterior Cruciate Ligament Reconstruction. Electric Stimulation. Whey Proteins.

RESUMO

Objetivo: Analisar os efeitos da estimulação elétrica neuromuscular do quadríceps femoral associado ou não à suplementação com whey protein na atividade eletromiográfica e distribuição de massa corporal em voluntários submetidos à reconstrução do ligamento cruzado anterior. Métodos: 24 voluntários foram divididos em três grupos: controle basal, whey protein associado com estimulação elétrica neuromuscular e estimulação elétrica neuromuscular isolada. Resultados: Na avaliação pós-operatória, durante o miniagachamento, o grupo controle basal demonstrou diminuição da atividade eletromiográfica do vasto medial (p = 0,005, olhos abertos; p = 0,003, olhos fechados), vasto lateral (p = 0,005, olhos abertos; p = 0,020, olhos fechados) e reto femoral (p = 0,075, olhos abertos; p = 0,074, olhos fechados) e da distribuição de massa corporal no membro operado (p < 0,001, olhos abertos; p < 0,001, olhos fechados) e membro lesionado (p < 0,001, olhos abertos; p < 0,001, olhos fechados). Conclusão: O uso precoce de estimulação elétrica neuromuscular do quadríceps femoral, independentemente do uso de whey protein, foi eficaz para manter a atividade eletromiográfica dos músculos vasto medial e vasto lateral, e prevenir assimetrias na distribuição de massa corporal 15 dias após a reconstrução do ligamento cruzado anterior. ***Nível de Evidência I, Ensaio randomizado de alta qualidade.***

Descritores: *Reconstrução do Ligamento Cruzado Anterior. Estimulação Elétrica. Proteínas do Soro do Leite.*

Citation: Mendonça GO, Severino MLB, Oliveira KM, Oliveira ML, Souza GG, Simão AP, Lobato DFM, Andrade RA, Franco RZ, Cruz MS, Carvalho LC. The effects of neuromuscular electrical stimulation in association with whey protein supplementation after anterior cruciate ligament reconstruction. Acta Ortop Bras. [online]. 2021;29(6):316-322. Available from URL: http://www.scielo.br/aob.

INTRODUCTION

Anterior cruciate ligament (ACL) rupture is a common lesion, occurring mainly in young people who participate in physical activity.[1,2] This lesion can be treated conservatively or through surgery.[3] The reconstructive surgery of this ligament is one of the more common orthopedic surgical procedures.[4,5,6]

All authors declare no potential conflict of interest related to this article.

The study was conducted at Federal University of Alfenas.
Correspondence: Gabriela Otília Mendonça. Av. Jovino Fernandes Sales, 2600, Alfenas, MG, Brazil, 37133840. gabriela.mendonca@unifal-mg.edu.br

Article received on 05/13/2020, approved on 10/15/2020.

Patients undergoing ACL reconstruction surgery have persistent atrophy and loss of quadriceps femoris strength.[7] The main factors for the dysfunction of this quadriceps muscle are the period of immobilization, disuse, decreased overload,[8] deficits in the ability to activate muscle fibers, muscle atrophy,[8] and muscle arthrogenic inhibition.[9]
Quadriceps arthrogenic muscle inhibition is an inhibitory reflex of the musculature of the knee region that occurs when there is joint damage, pain, edema and inflammation.[10] This inhibition is considered a protective mechanism for avoiding injury; however, this same inhibition may limit rehabilitation.[11] Interventions aimed at improving the voluntary activation of the quadriceps femoris are important because the early restoration of quadriceps femoris strength positively influences the following phases of rehabilitation.[12]
In this context, one effective and safe intervention that can be used early to improve quadriceps femoris strength is neuromuscular electrical stimulation (NMES).[9,13] In patients undergoing ACL reconstruction, NMES can be used to decrease arthrogenic muscle inhibition, help restore strength and minimize quadriceps femoris atrophy.[9,13,14]
To help maintain muscle mass, muscle stimulation is very important, but dietary intake should also be considered, especially protein intake, which stimulates the synthesis of muscle proteins.[15] Therefore, one intervention that may be used in association with NMES to help minimize strength loss and muscular atrophy due to short-term disuse is the administration of whey protein.
Whey protein is a nutritional supplement rich in essential amino acids, which are important for assisting in the stimulation of muscle protein synthesis and thus improve muscle strength and aid in the muscle hypertrophy process.[16] However, its use is not exclusive to those who are seeking muscular hypertrophy and its effect in regard to rehabilitation has only recently been studied.[17,18]
The aim of this study was to analyze the electromyographic activity of the rectus femoris, vastus lateralis and vastus medialis muscles and body mass distribution during a bipodal mini squat movement in volunteers who had undergone ACL reconstruction surgery and had taken NMES of the quadriceps femoris associated or not with whey protein. The authors hypothesized that the group receiving the whey protein intervention in association with NMES would obtain better results than the other groups of the study.

METHODS

This is a controlled randomized, blinded clinical trial that was approved by the Research Ethics Committee for research on human beings at the Federal University of Alfenas (opinion number: 1.940.399). This study was also registered in the Brazilian clinical trial registry (REBEC). Before participating in the study, all volunteers signed an informed consent form.
The volunteers were recruited at orthopedic clinics located in Alfenas-MG. Participants were recruited from October 2016 to June 2018. Eligibility candidates were those who presented rupture of the ACL and who were scheduled to undergo ACL reconstruction surgery. The inclusion criteria were: male volunteers aged 18 to 50 who presented a unilateral rupture of the ACL as verified by magnetic resonance imaging and who were scheduled to undergo ACL reconstruction. The exclusion criteria were: those with a history of lower limb surgery, renal disease, lactose intolerance or diabetes. After an interview, the block randomization of eight volunteers was performed using a random number application by a trained researcher who was responsible for the interventions. Other researchers were responsible for assessing and analyzing the data early on when volunteers were allocated to their respective groups. However, due to the nature of the interventions, it was not possible to blind the therapist and the patients.
The sample consisted of 24 male volunteers who were divided into three groups: basal control (BC), whey protein supplementation in association with the NMES of the quadriceps femoris (WE), and the NMES of the quadriceps femoris alone (ES). The BC group was composed of volunteers who participated in evaluations only twice: a preoperative evaluation and a reassessment 15 days after surgery. The WE group was composed of volunteers who underwent both NMES and whey protein supplementation, taking a dose of 20 grams of whey protein in 250 ml of water as a vehicle. The ES group consisted of volunteers who underwent the NMES of the quadriceps femoris with only 250 ml of preintervention water administered. One researcher was responsible for performing the NMES intervention and giving whey protein or water to the volunteers. All groups were verbally oriented for immediate postoperative care, such as the use of cryotherapy and the initiation of gradual weight-bearing where the surgery was performed.
All groups underwent two evaluations performed at two different periods: a prior evaluation, approximately 7 days before the surgery (preoperative); and a reevaluation, 15 days after the surgical procedure (postoperative). For all groups, the evaluations were performed using an evaluation card, surface electromyography (sEMG) and baropodometry.

Evaluation procedures

Initially, an evaluation form was used where data such as age, body mass, height, body mass index (BMI) and mean time of injury were collected.

Surface electromyography (sEMG)

The sEMG is a non-invasive method for assessing skeletal muscle activity that verifies the electrical power of muscles.[19] This method can be used to evaluate the effectiveness of rehabilitation or physical exercise.[20]
For the electromyographic evaluation of the rectus femoris, vastus medialis and vastus lateralis muscles, the Trigno 8 Channel Wireless device (EMGworks, Delsys Inc., Boston, MA, USA) equipped with EMGworks 4.0 acquisition software was used.
The mode of acquisition of the electromyographic signals was calibrated at a sampling frequency of 1000 Hz with 1000-fold gain, a 20 Hz high-pass filter, 500 Hz low-pass filter and 60 Hz filter to prevent grid interference.
To reduce possible interference in the acquisition of the electromyographic signal, trichotomy and the cleaning of the skin with 70% alcohol were performed in the areas where the electrodes were placed, which were fixed to the volunteers' skin with double-sized tape. The electrodes were placed on the muscles as recommended by the European Society of Surface Electromyography (SENIAM).[21]
The electromyographic evaluation of the muscles was performed during a maximum voluntary isometric contraction (MVIC) and during a bipodal mini squat with eyes open and posteriorly with eyes closed. Five acquisitions were made for each condition lasting six seconds each and, between each collection, a 30-second interval was inserted. The electromyographic signal for the MVIC collected from the previously mentioned musculatures was taken against manual resistance, which was performed each time by the same evaluator. For the electromyographic collection of the MIVC, joint position was standardized for all volunteers, and they were seated with a 60-degree knee flexion.[22]
After the collection of the MVIC, data on the bipodal mini squat were collected. The volunteers were instructed how to perform the bipodal mini squat to reach a 30-degree knee flexion, and this angulation was demonstrated using a goniometer.
For the analysis and interpretation of the records, EMGWorks 4.0 analysis software was used to obtain root mean square (RMS) and peak parameters, which excluded the first and last second of the collection for a total of four seconds.
Gross sEMG data are often sensitive to a number of extrinsic and intrinsic factors.[23,24] Thus, the interpretation of gross sEMG data is subject to different challenges, indicating the need for a normalization process for these data.[22] For the normalization of the sEMG data during the bipodal mini squat, the value during the squat was considered with an sEMG reference value from the MVIC of same muscle, which was as follows:[25]

$$\text{Normalization} = \frac{\text{RMS bipodal mini squat}}{\text{RMS of MVIC}} \times 100$$

Baropodometry

A FootWork Pro baropodometer (IST-Informatique, France) was used to observe the distribution of body mass during the bipodal mini squat with eyes open and closed.

The volunteers were positioned in orthostatism on the platform with their feet equidistant. Static balance was assessed with eyes open and gaze focused on a wall two meters away to the front. Then, the mini squat movement was performed. Five repetitions were done with eyes open and then five with eyes closed. The collection time for each repetition was six seconds and, between each collection, a 30-second interval was given to avoid possible fatigue. The body mass data were acquired at an acquisition frequency of 100 Hz and then analyzed by the software FootWork Pro v. 3.2.2.0 (IST-Informatique, France). Body mass distribution was analyzed on the anterior, posterior and injured and healthy limb regions as shown in Figure 1.

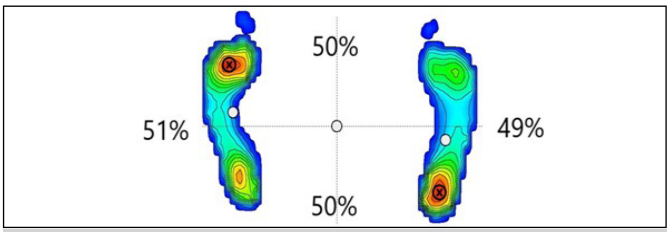

Figure 1. Distribution of body mass in the anterior, posterior, injured and healthy limbs.

Neuromuscular electrical stimulation (NMES)

For NMES, the Neurodyn High Volt device from Ibramed was used. The procedure was performed individually under the supervision of a trained researcher. The following parameters were used: a frequency of 50 Hz, 4-second rise time, 4-second descent time, ton of 5, and toff of 15. The current intensity in milliamperes was initially adjusted to the maximum tolerance of the individual that produced a contraction, which was increased according to the accommodation of the current. The total application time was 20 minutes. All volunteers performed three weekly applications with a one-day interval between for two consecutive weeks for a total of six procedures. The volunteers were seated, with a 90-degree flexion of the hips and knees. Four electrodes were placed on the quadriceps femoris muscle and two proximal and two distal to the muscle were positioned according to the previous location of the motor points of the vastus medialis and vastus lateralis muscles.

Whey protein supplementation

In total, 20 grams of whey protein of the Hilmar Ingredients® brand was dissolved in 250 ml of water. The volunteers ingested the whey protein after the NMES intervention. This 20-gram dose of whey protein is sufficient to stimulate the synthesis of muscle proteins in young individuals.[26,27]

Statistical analysis

The statistical analysis of the data was performed using SPSS software (IBM Corp., Chicago, IL, USA), version 20.0.

Initially the data were analyzed with descriptive statistical methods, obtaining values for mean and standard deviation. All data sets were tested for their normality using Shapiro-Wilk tests.

Then the variables of age, body mass, height, BMI and time of injury were analyzed using a one-way analysis of variance (ANOVA) test. For the categorical variable, the type of graft, a chi-square test was used. The other data from the study were submitted to a general linear model procedure using the repeated measures of ANOVA test followed by a Bonferroni test to verify the interaction between the groups (BC, WE and ES) with the pre- and postoperative evaluations. A 5% significance level was considered for all analyses. To calculate the effect size in ANOVA, f^2 Cohen was used as well as values from 0.02 to 0.15 (small effect), 0.15 to 0.35 (median effect), and above 0.35 (large effect).

RESULTS

Between October 2016 and June 2018, 47 volunteers were assessed for eligibility. Of these, 23 were excluded. The reasons for exclusion were being female (n = 3), over 50 years old (n = 2), less than 18 years old (n = 2), having a history of lower limb surgery (n = 3) and living in a different city (n = 13). At the end of the selection, 24 volunteers fulfilled the selection criteria and accepted to participate in the study. Then they were randomized and received an allocation into one of the three research groups. Figure 2 illustrates the flowchart of the study.

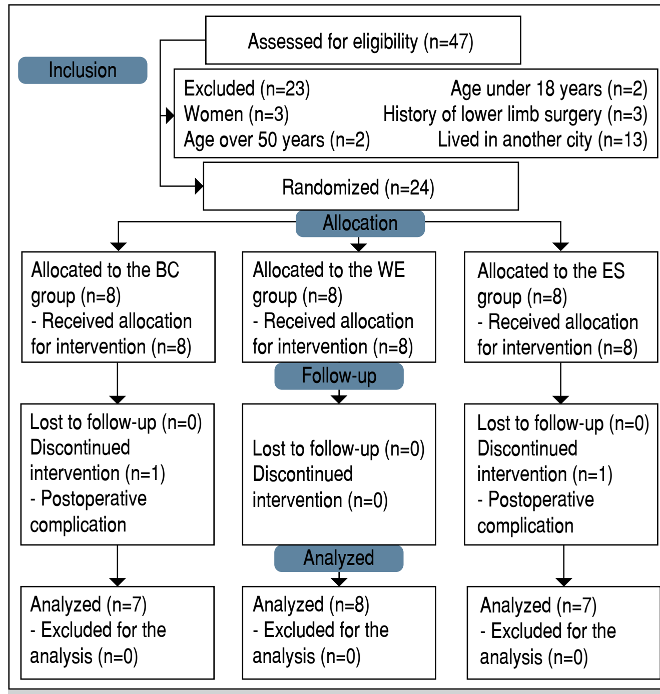

Figure 2. Abbreviations: BC, basal control group; WE, whey protein and neuromuscular electrical stimulation (NMES) group. ES, NMES group.

Table 1 shows data related to age, anthropometric characteristics, time of injury and the type of graft used in the surgeries. At the beginning of the study, we observed there were no significant differences between the groups for these variables.

Table 1. Mean values ± standard deviation of age, body mass, height, body mass index (BMI), time of injury, and type of graft used in the different study groups.

Variable		BC (n = 7)	WE (n = 8)	ES (n = 7)	P Value
Age (years)		27.71 ± 6.73	35.13 ± 8.77	32.29 ± 9.14	0.487
Body Mass (kg)		75.51 ± 4.96	85.50 ± 6.65	77.36 ± 10.50	0.070
Stature (m)		1.73 ± 0.05	1.79 ± 0.07	1.76 ± 0.05	0.180
BMI (kg/m²)		25.32 ± 2.12	26.79 ± 1.41	25.18 ± 3.71	0.226
Mean Time of Injury(months)		10.71 ± 8.69	7.25 ± 6.25	13.00 ± 16.85	0.621
Type of Graft	GF(%)	5 (71.42%)	7 (87.5%)	6 (85.71%)	0.686
	GP(%)	2 (28.58%)	1 (12.5%)	1 (14.83%)	

Abbreviations: BC, basal control group; WE, whey protein and neuromuscular electrical stimulation (NMES) group; ES, NMES group; GF, graft with flexor tendon; GP, graft with patellar tendon.

Table 2 shows the normalized data from the electromyographic evaluation of the muscles in the injured lower limb during the bipodal mini squat with eyes open and closed.

Table 2. Normalized electromyographic analysis of the muscles of the injured knee during the bipodal mini squat with eyes open (EO) and closed (EC) among the different groups of the study in the pre- and postoperative evaluations.

Variable	Groups	PRE	POST	ANOVA p value			f^2
				Evaluation	Group	Evaluation*Group	
VM-EO	BC	45.85 ± 25.32*	16.37 ± 13.57				
	WE	46.32 ± 23.45	32.86 ± 20.26	0.005	0.506	0.435	0.342
	ES	50.96 ± 39.66	32.02 ± 27.10				
VL-EO	BC	52.42 ± 41.20*	23.30 ± 16.39				
	WE	44.19 ± 24.92	31.55 ± 19.07	0.005	0.718	0.362	0.343
	ES	54.75 ± 30.03	35.31 ± 26.78				
RF-EO	BC	55.01 ± 31.68	34.64 ± 25.29				
	WE	57.39 ± 49.28	55.72 ± 47.70	0.075	0.146	0.349	0.157
	ES	26.11 ± 16.03	18.96 ± 11.76				
VM-EC	BC	44.80 ± 22.65*	16.37 ± 13.57				
	WE	44.82 ± 22.17	29.98 ± 21.71	0.003	0.251	0.526	0.385
	ES	50.62 ± 38.46	33.14 ± 25.96				
VL-EC	BC	45.72 ± 41.43*	23.53 ± 16.28				
	WE	44.72 ± 29.60	31.36 ± 19.09	0.020	0.559	0.722	0.254
	ES	53.87 ± 30.63	35.25 ± 26.82				
RF-EC	BC	55.28 ± 37.60	30.49 ± 26.75				
	WE	38.90 ± 28.84	35.43 ± 20.15	0.074	0.826	0.256	0.308
	ES	44.89 ± 33.03	21.54 ± 16,92				

Abbreviations: f^2, effect size; VM, vastus medialis muscle; BC, basal control; WE, group that received whey protein in association with neuromuscular electrical stimulation (NMES) in the femoral quadriceps; ES, group that received only NMES in the femoral quadriceps; VL, vastus lateralis muscle; RF, rectus femoris muscle; * versus pre-evaluation, differs significantly based on the Bonferroni test ($p < 0.05$).

Based on these results, it is possible to verify that during the bipodal mini squat with eyes open, the BC group showed a decrease in the electromyographic activity of the vastus medialis ($p = 0.005$) and vastus lateralis ($p = 0.005$) muscles as compared to the initial evaluation. In the closed-eye analysis, the BC group also had a decrease in the electromyographic activity of the vastus medialis ($p = 0.003$) and vastus lateralis ($p = 0.020$) muscles in the postoperative evaluation as compared to the initial evaluation. The WE and ES groups did not demonstrate a significant decrease in the electromyographic activity of the vastus medialis and vastus lateralis muscles. It is possible to infer that NMES was effective in maintaining the electromyographic activity of these muscles during the mini squat movement. The other variables did not show significant differences.

Table 3 shows the normalized data from electromyographic evaluation of the muscles of the healthy lower limb during the bipodal mini squat with eyes open and closed. It can be observed there were no significant differences in the electromyographic activity of the evaluated muscles of the healthy limbs.

Table 3. Normalized electromyographic analysis of muscles of the healthy lower limb during the bipodal mini squat with eyes open (EO) and closed (EC) among the different groups of the study in the pre- and postoperative evaluations.

Variable	Groups	PRE	POST	ANOVA p value			f^2
				Evaluation	Group	Evaluation*Group	
VM-EO	BC	34.85 ± 24.34	41.15 ± 22.68				0.182
	WE	46.97 ± 21.48	59.12 ± 40.01	0.053	0.647	0.377	
	ES	39.99 ± 26.77	56.22 ± 29.47				
VL-EO	BC	38.12 ± 25.00	41.98 ± 26.34				
	WE	48.26 ± 23.40	54.54 ± 24.32	0.250	0.547	0.259	0.069
	ES	36.66 ± 26.47	53.31 ± 25.62				
RF-EO	BC	48.76 ± 43.49	73.53 ± 64.01				
	WE	22.44 ± 13.14	38.79 ± 25.68	0.054	0.074	0.831	0.202
	ES	23.83 ± 13.67	30.98 ± 20.63				
VM-EC	BC	34.72 ± 25.81	43.55 ± 21.29				
	WE	48.28 ± 30.21	51.72 ± 35.44	0.087	0.777	0.241	0.241
	ES	35.06 ± 31.09	54.58 ± 30.04				
VL-EC	BC	42.85 ± 30.90	39.47 ± 19.84				
	WE	48.11 ± 25.59	52.03 ± 25.62	0.211	0.721	0.118	0.086
	ES	27.97 ± 19.27	54.65 ± 35.04				
RF-EC	BC	29.41 ± 15.24	37.43 ± 18.25				
	WE	36.62 ± 34.24	42.46 ± 31.11	0.080	0.618	0.685	0.160
	ES	28.81 ± 15.51	46.97 ± 35.95				

Abbreviations: f^2, effect size; VM, vastus medialis muscle; BC, basal control; WE, group that received whey protein in association with neuromuscular electrical stimulation (NMES) in the femoral quadriceps; ES, group that received only NMES in the femoral quadriceps; VL, vastus lateralis muscle; RF, rectus femoris muscle.

Table 4 shows data regarding body mass distribution during the bipodal mini squat with eyes open and closed.

Table 4. Body mass distribution (%) of the injured and healthy limbs during the bipodal mini squat with eyes open (EO) and closed (EC) among the different groups of the study in the pre- and postoperative evaluations.

Variable	Groups	PRE	POST	ANOVA p value			f^2
				Evaluation	Group	Evaluation*Group	
IL-EO	BC	54.57 ± 6.90*	36.83 ± 11.62	< 0.001	0.178	0.533	0.610
	WE	45.60 ± 5.22	34.32 ± 8.77				
	ES	46.34 ± 5.68	34.45 ± 10.92				
HL-EO	BC	45.53 ± 6.90*	63.17 ± 11.62	< 0.001	0.173	0.499	0.603
	WE	54.92 ± 6.05	65.67 ± 8.77				
	ES	53.65 ± 5.68	65.54 ± 10.92				
ANT-EO	BC	46.96 ± 8.91	44.09 ± 12.64	0.334	0.002	0.781	0.049
	WE	61.60 ± 4.68†	58.37 ± 6.20†				
	ES	52.65 ± 8.64	52.68 ± 6.44				
POS-EO	BC	53.34 ± 8.91	55.91 ± 12.64	0.353	0.002	0.788	0.045
	WE	38.40 ± 4.68†	41.62 ± 6.20†				
	ES	47.34 ± 6.50	47.31 ± 6.44				
IL-EC	BC	53.62 ± 7.00*	37.20 ± 11.54	<0.001	0.312	0.653	0.594
	WE	48.00 ± 7.80	35.25 ± 9.35				
	ES	45.31 ± 6.50	34.68 ± 9.95				
HL-EC	BC	46.38 ± 7.00*	62.80 ± 11.54	< 0.001	0.694	0.677	0.690
	WE	52.00 ± 7.80	64.75 ± 9.35				
	ES	54.68 ± 6.50	65.60 ± 10.06				
ANT-EC	BC	50.25 ± 9.82	45.48 ± 11.42	0.224	0.023	0.787	0.077
	WE	60.07 ± 6.06†	58.15 ± 7.62†				
	ES	54.51 ± 9.28	53.20 ± 7.43				
POS-EC	BC	49.75 ± 9.82	54.52 ± 11.42	0.135	0.015	0.789	0.114
	WE	39.25 ± 5.45†	41.85 ± 7.56†				
	ES	45.58 ± 9.28	46.80 ± 7.43				

Abbreviations: f^2, effect size; BC, basal control group, WE, whey protein and neuromuscular electrical stimulation (NMES) group. ES, NMES group. IL; mass on the injured limb; HL, mass on the healthy limb; ANT, mass in the anterior region; POS, mass in the posterior region;
* versus pre-evaluation, differ significantly based on the Bonferroni test ($p < 0.05$).
† significant difference in relation to the BC group based on the Bonferroni test ($p < 0.05$).

Considering the results, we can observe that the volunteers in the BC group experienced a decrease in the body mass distribution of the injured limb during the bipodal mini squat with eyes open ($p < 0.001$) and closed ($p < 0.001$) in the postoperative evaluation as compared to the preoperative evaluation. This group also showed an increased body mass distribution in the healthy limb during the bipodal mini squat both with eyes open ($p < 0.001$) and closed ($p < 0.001$). The WE and ES groups showed no asymmetries in body mass distribution between the injured and healthy limbs, thus it is possible to infer that NMES was effective in treating this variable.

We observed in the pre- and postoperative evaluations that the WE and CB groups showed differences in the body mass distribution of the anterior and posterior regions during the bipodal mini squat with eyes open and closed. As compared to the BC group, the WE group showed a greater body mass distribution in the anterior region during the bipodal mini squat with eyes open ($p = 0.002$) and closed ($p = 0.023$) in the preoperative and postoperative evaluations and a lower body mass distribution in the posterior region during the bipodal mini squat with eyes open ($p = 0.002$) and closed ($p = 0.015$). Thus, we can verify that, for the anterior and posterior variables, the BC and WE groups differed in both evaluations.

DISCUSSION

This study evaluated the effects of the use of whey protein supplementation in association with NMES of the quadriceps femoris in patients who underwent ACL reconstruction surgery. Our hypothesis is that the group receiving the whey protein supplementation in association with NMES would obtain better results, since such interventions would stimulate muscle protein synthesis.

There are few studies that have considered the synergism use of NMES and protein supplementation. In the literature, only two studies considering these interventions together were found,[18,28] but neither evaluated the effect of NMES alone. Zange et al.[28] found that NMES in the soleus muscle in association with whey protein supplementation was effective in preserving leg muscle volume and, to a lesser extent, in maintaining the strength of the plantar flexors in healthy individuals who had used a brace in one leg 8–16 hours per day. Reidy et al.[18] found that NMES and protein supplementation were able to maintain lean mass, but there was no attenuation in the decline of muscle function and strength in resting older patients who remained in bed for five days. Although these studies have used interventions similar to those of our work, a comparison of the results is difficult due to differences in methodology and target populations.

Weakness of the quadriceps femoris muscle is a factor that negatively influences knee function and pain.[29] During the bipodal mini squat with eyes open, we observed that the volunteers of the BC group had a significant decrease in the electromyographic activity of the vastus medialis and vastus lateralis muscles of the injured limb, which did not occur in the other study groups. The use of NMES in association with rehabilitation during the initial phase after ACL reconstruction is effective in preventing atrophy,[9,14] reducing loss of muscle strength in the quadriceps femoris,[9,13,24] assisting in strength recovery and quadriceps femoris symmetry,[14] and decreasing muscle inhibition.[11,9] NMES has been effective in maintaining the electromyographic activity of the vastus medialis and vastus lateralis muscles during voluntary activities such as the bipodal mini squat since it is capable of acting as a disinhibitory and activating tool for the quadriceps femoris muscle.[11]

Although there are many studies related to the use of NMES in patients after ACL reconstruction, there has been a great variability in the parameters and protocols used, making it difficult to compare results.[9,13] However, most studies agree on the intensity used, which has been described as the maximum that may be tolerated by the patient.[9,13] Thus, this parameter was adopted in this study.

The evaluation of body mass distribution in dynamic movements provides important information about the asymmetry of loads in injured and healthy limbs.[30] In the analysis of the bipodal mini squat with eyes open and closed, the BC group demonstrated a higher concentration of mass on the healthy limb and a consequent decrease in the injured limb. We believe = this compensatory mechanism was due to kinesiophobia, but we did not evaluate this variable. Other groups did not present this same mechanism. It is probable that NMES alone or in association with whey protein supplementation favored the obtained responses since it is capable of acting as a source of disinhibition by restoring the function of the quadriceps femoris muscle.[11]

When analyzing the anterior and posterior body mass concentrations of the volunteers, we observed that those belonging to the BC and WE groups differed significantly in both evaluations. This may have occurred due to the randomization process adopted by the study randomly selecting subjects with higher body mass distribution concentrations in the anterior region for the WE group. It is important to emphasize that body mass distribution should be established as soon as possible, as patients undergoing ACL reconstruction surgery tend to show a reduction in bone mineral density in the hip region, and this is a risk factor for other lesions.[31]

This study has some limitations that should be considered. First, it has a relatively small sample, so care must be taken when interpreting and generalizing the data. Another limitation is that there was no diet control for the volunteers. The dose and frequency of protein supplementation may also be considered a limitation. In addition, it was not possible to blind the volunteers.

CONCLUSION

In conclusion, in the sample of this study, we observed the early use of NMES of the quadriceps femoris muscle, regardless of whey protein supplementation, maintained the electromyographic activity of the vastus medialis and vastus lateralis muscles of the injured limb during the mini squat movement and prevented asymmetries in body mass distribution approximately 15 days after ACL reconstruction surgery.

ACKNOWLEDGMENTS

The authors wish to thank the patients who participated in the study, Tutorial Education Program (PET-MEC-SESU) and Federal University of Alfenas. This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001 and the Graduate Program in Rehabilitation Science of the Federal University of Alfenas – UNIFAL-MG.

AUTHORS' CONTRIBUTIONS: Each author contributed individually and significantly to the development of this article. GOM: data analysis and writing of the article; MLBS: data acquisition and interventions; KMO: data acquisition and interventions; MLO: writing of the article and data analysis; GGS: contribution to the study design and final approval of the manuscript; APS: critical content revision and final approval of the manuscript; DFML: critical content revision and final approval of the manuscript; RAA: surgeries and approval of the final version of the manuscript; RZF: surgeries and approval of the final version of the manuscript; MSC: surgeries and final approval of the manuscript; LCC: contribution to the study design and critical content revision.

REFERENCES

1. Łyp M, Stanis awska I, Witek B, Majerowska M, Czarny-Dzia ak M, W ostowska E. The timing of rehabilitation commencement after reconstruction of the anterior cruciate ligament. Adv Exp Med Biol. 2018;1096:53-7.
2. Monk AP, Davies LJ, Hopewell S, Harris K, Beard DJ, Price AJ. Surgical versus conservative interventions for treating anterior cruciate ligament injuries (Review). Cochrane Database Syst Rev. 2016;4:1-38.
3. Moksnes H, Risberg, MA. Performance-based functional evaluation of non--operative and operative treatment after anterior cruciate ligament injury. Scand J Med Sci Sports. 2009;19(3):345-55.
4. Ichiba A, Tokuyama F, Makuya K, Oda K. Graft quality and clinical outcomes of intraoperative bone tunnel communication in anatomic double-bundle anterior cruciate ligament reconstruction. Asia Pac J Sports Med Arthrosc Rehabil Technol. 2016;3:6-12.
5. Oliveira VM, Latorre GC, Netto AS, Jorge RB, Hernandez Filho G, Curry RPL. Estudo da relação entre a espessura do ligamento cruzado anterior, os dados antropométricos e as medidas anatômicas do joelho. Rev Bras Ortop. 2016;51(2):194-9.
6. Vinagre G, Kennedy NI, Chahla J, Hussain ZB, Olesen ML, LaPrade RF, et al. Hamstring graft preparation techniques for anterior cruciate ligament reconstruction. Arthrosc Tech. 2017;6:e2079-84.
7. Palmieri-Smith RM, Thomas AC, Wojtys EW. Maximizing quadriceps strength after ACL reconstruction. Clin Sports Med. 2008;27(3):405-24.
8. Žargi TG, Drobni M, Vauhnik R, Koder J, Kacin A. Factors predicting quadriceps femoris muscle atrophy during the first 12 weeks following anterior cruciate ligament reconstruction. Knee. 2017;24(2):319-28.
9. Hauger AV, Reiman MP, Bjordal JM, Sheets C, Ledbetter L, Goode AP. Neuromuscular electrical stimulation is effective in strengthening the quadriceps muscle after anterior cruciate ligament surgery. Knee Surg Sports Traumatol Arthrosc. 2017;26(2):399-410.
10. Hopkins JT, Ingersoll CD. Arthrogenic muscle inhibition: a limiting factor in joint rehabilitation. J Sport Rehabil. 2000;9(2):135-59.
11. Harkey MS, Gribble PA, Pietrosimone BG. Disinhibitory interventions and voluntary quadriceps activation: a systematic review. J Athl Train. 2014;49(3):411-21.
12. Saka T. Principles of postoperative anterior cruciate ligament rehabilitation. World J Orthop. 2014;5(4):450-9.
13. Kim K-M, 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.
14. Labanca L, Rocchi JE, Laudani L. Neuromuscular electrical stimulation superimposed on movement early after ACL surgery. Med Sci Sports Exerc. 2017;50(3):407-16.
15. Dirks ML, Groen BBL, Franssen R, van Kranenburg J, van Loon LJC. Interventional strategies to combat muscle disuse atrophy in humans: focus on neuromuscular electrical stimulation and dietary protein. J Appl Physiol. 2018;125(3):850-61.
16. Devries MC, Phillips SM. Supplemental protein in support of muscle mass and health: Advantage whey. J Food Sci. 2015;80:A8-A15.
17. Niitsu M, Ichinose D, Hirooka T. Effects of combination of whey protein intake and rehabilitation on muscle strength and daily movements in patients with hip fracture in the early postoperative period. Clin Nutr. 2016;35(4):943-9.
18. Reidy PT, McKenzie AI, Brunker P, Nelson DS, Barrows KM, Supiano M, et al. Neuromuscular electrical stimulation combined with protein ingestion preserves thigh muscle mass but not muscle function in healthy older adults during 5 days of bed rest. Rejuvenation Resh. 2017;20(6):449-61.
19. Merletti R, Parker PA. Electromyography: physiology, engineering and noninvasive applications. Piscataway: IEEE Press; 2004.
20. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol. 2002;92:230918.
21. Hermens HJ, Freriks B, Merletti R. European recommendations for surface electromyography. Results of the SENIAM Project. Enschede: Roessingh Research and Development; 1999.
22. Albertus-Kajee Y, Tucker R, Derman W, Lamberts RP, Lambert MI. Alternative methods of normalising EMG during running. J Electromyogr Kinesiol. 2011;21(4):579-86.

23. De Luca CI. The use of surface electromyography in biomechanics. J Appl Biomater Biomech. 1997; 13:135-63.
24. Hasegawa S, Kobayashi M, Arai R, Tamaki A, Nakamura T, Moritani T. Effect of early implementation of electrical muscle stimulation to prevent muscle atrophy and weakness in patients after anterior cruciate ligament reconstruction. J Electromyogr Kinesiol. 2011;21(4):622-30.
25. Halaki M, Ginn K. Normalization of EMG signals: to normalize or not to normalize and what to normalize to? London: IntechOpen; 2012.
26. Akhavan T, Luhovyy BL, Panahi S, Kubant R, Brown PH, Anderson GH. Mechanism of action of pre-meal consumption of whey protein on glycemic control in young adults. J Nutr Biochem. 2014;25(1):36-43.
27. Morton RW, Mcglory C, Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. Front Physiol. 2015;6:1-9.
28. Zange J, Schopen K, Albracht K. Using the hephaistos orthotic device to study countermeasure effectiveness of neuromuscular electrical stimulation & dietary lupin protein supplementation, a randomised controlled trial. PLos One. 2017;12:1-13.
29. Schmitt LC, Paterno MV, Hewett TE. The impact of quadriceps femoris strength asymmetry on functional performance at return to sport following anterior cruciate ligament reconstruction. J Orthop Sports Phys Ther. 2012;42(9):750-9.
30. Chmielewski TL. Asymmetrical lower extremity loading after ACL reconstruction: more than meets the eye. J Orthop Sports Phys Ther. 2011;41(6):374-6.
31. Reiman MP, Rogers ME, Manske RC. Interlimb differences in lower extremity bone mineral density following anterior cruciate ligament reconstruction. J Orthop Sports Phy Ther. 2006;36(11):837-44.