Modified hybrid training and neuromuscular electrical stimulation increase quadriceps femoral and hamstrings muscle hypertrophy in untrained healthy subjects

El Entrenamiento híbrido modificado y la estimulación eléctrica neuromuscular aumentan la hipertrofia muscular de los cuádriceps femorales e isquiotibiales en sujetos sanos no entrenados

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Abstract

Purpose: Low physical activity leads to muscle atrophy due to lack of exercise time and sport facilities. Addressing this issue, there are alternative methods with shorter training time and less equipment to induce muscle hypertrophy which are neuromuscular electrical stimulation (NMES) Russian protocol and modified hybrid training. We analyzed the difference in muscle hypertrophy of quadriceps and hamstrings between these two methods in untrained healthy subjects.

Methods: This study was an experimental study on 22 untrained healthy men aged 18-40 which were randomly assigned to the hybrid and NMES group. There were 11 people in each group undergoing the intervention three times a week for 4 weeks. At the beginning and end of the intervention, muscle thickness was measured by B-Mode ultrasonography. The results were compared between groups.

Results: There was a significant increase in muscle hypertrophy in the hybrid group, which are quadriceps (dominant leg p=0.019, non-dominant leg p=0.007) and hamstrings (dominant leg p = 0.013, non-dominant leg p= 0,002). In the NMES group, a significant increase was found in the quadriceps muscle (dominant leg p=0.011, non-dominant leg p=0.002). There was a significant difference in the change of hamstring muscle thickness on the non-dominant leg (p=0.004) between the two groups.

Conclusion: Modified hybrid training and NMES Russian protocol were shown to increase quadriceps and hamstring hypertrophy after 4 weeks intervention. In addition, modified hybrid training was shown to increase non-dominant side hamstring muscle hypertrophy greater than NMES Russian protocol.

Keywords

Hybrid Training System; Muscle Hypertrophy; Russian stimulation; Quadricep; Hamstring

Resumen

Propósito: La baja actividad física conduce a la atrofia muscular debido a la falta de tiempo para el ejercicio y de instalaciones deportivas. Para abordar este problema, existen métodos alternativos con menor tiempo de entrenamiento y menos equipamiento para inducir hipertrofia muscular, como la estimulación eléctrica neuromuscular (NMES) con protocolo ruso y el entrenamiento híbrido modificado. Analizamos la diferencia en la hipertrofia muscular de los cuádriceps e isquiotibiales entre estos dos métodos en sujetos sanos no entrenados.

Métodos: Este estudio experimental se realizó en 22 hombres sanos no entrenados de 18 a 40 años, asignados aleatoriamente al grupo de entrenamiento híbrido y al grupo de NMES. Cada grupo incluyó a 11 personas que realizaron la intervención tres veces por semana durante 4 semanas. Al inicio y al final de la intervención, se midió el grosor muscular mediante ultrasonografía en modo B. Los resultados se compararon entre los grupos.

Resultados: Hubo un aumento significativo en la hipertrofia muscular en el grupo de entrenamiento híbrido, tanto en los cuádriceps (pierna dominante p=0.019, pierna no dominante p=0.007) como en los isquiotibiales (pierna dominante p=0.013, pierna no dominante p=0.002). En el grupo de NMES, se encontró un aumento significativo en los cuádriceps (pierna dominante p=0.011, pierna no dominante p=0.002). Hubo una diferencia significativa en el cambio de grosor muscular de los isquiotibiales en la pierna no dominante (p=0.004) entre los dos grupos.

Conclusión: El entrenamiento híbrido modificado y el protocolo ruso de NMES demostraron aumentar la hipertrofia de los cuádriceps y los isquiotibiales después de una intervención de 4 semanas. Además, el entrenamiento híbrido modificado mostró un mayor aumento en la hipertrofia de los isquiotibiales del lado no dominante en comparación con el protocolo ruso de NMES.

Palabras clave

Hybrid Training System; Hipertrofia Muscular, Russian stimulation, cuádriceps, isquiotibiales.

Introduction

Low physical activity contributes to muscle atrophy, which is associated with reduced muscle strength, impaired physical function, and a higher risk of falls (Ghozy et al., 2021; Kristiana et al., 2020). Muscle thickness, an indicator of muscle mass, is commonly measured using ultrasonography (USG) due to its ease of use and high reliability (Thoirs & English, 2009). Strength training is a widely recognized and thoroughly studied approach to address muscle atrophy by enhancing muscle hypertrophy, typically through resistance exercises such as weightlifting (Hasni et al, 2019). However, logistical challenges such as gym access and home equipment availability often discourage adherence to any exercise regimen (Riskawati et al., 2018). Thus, there is a need for practical and accessible approaches to enhance muscle hypertrophy. Neuromuscular electrical stimulation (NMES) offers an alternative solution. By delivering electrical impulses to motor nerves, NMES induces muscle contractions, effectively preventing atrophy, strengthening muscles, and promoting hypertrophy (Mohammadi, 2024). NMES has been shown to increase muscle mass. The specificity theory posits that NMES preferentially activates fast-twitch muscle fibers, which are more susceptible to atrophy and capable of generating greater force (Cameron, 2018). NMES protocols, such as the Russian Protocol, utilize medium- to high-frequency electrical stimulation to improve muscle strength, with reports of strength gains up to 40% (Prabha et al., 2019; Prentice, 2009).

Another novel approach is the hybrid training system (HTS), which combines NMES with voluntary muscle contraction. HTS employs electrical stimulation of antagonist muscles to provide resistance during agonist contractions, thereby promoting muscle hypertrophy and functional gains (Rabe et al., 2018). Developed by researchers at Kurume University, HTS uses a unique stimulation device that coordinates antagonist stimulation with agonist contractions, targeting both fast- and slow-twitch muscle fibers (Tsukada et al., 2018). HTS uses Russian stimulation, which is a burst wave with a basic frequency of 5000 Hz delivered at 20 Hz (2.4 msec on, 47.6 msec off) in a rectangular, biphasic waveform (Rabe et al., 2018; Tsukada et al., 2018). HTS effectively evoking fast-twitch contractions during electrical eccentric contractions and evoking slow-twitch contractions during voluntary muscle contractions (Matsuse et al., 2024). Despite its efficacy, HTS requires specialized equipment, limiting its widespread use (Matsuse et al., 2024). We adapted the way HTS works by modifying it using electrical stimulation devices and other equipment found in our rehabilitation center and termed it modified hybrid training (MHT). This form of exercise may serve as a viable choice for muscle hypertrophy due to its brief duration and absence of necessity for resistance equipment. However, there are a variety of subject characteristics that could be confounding variables in this study, which are physical activity in daily living activities, nutritional intake, leg dominance, and body mass index (BMI).

No studies have directly compared the effects of MHT and the NMES Russian Protocol on quadriceps and hamstring muscle hypertrophy or examined potential differences between dominant and non-dominant legs. This study aims to evaluate the effects of MHT and NMES on muscle hypertrophy in untrained healthy subjects, addressing a critical gap in the literature.

Method

Study Design

This research is a randomized pre-test and post-test group design. The study participants were healthy, untrained men in the age range of 18 to 40 years who were part of the Medical Rehabilitation Installation at Dr. Soetomo Hospital Surabaya, Indonesia. They fulfilled all inclusion requirements and none of the exclusion criteria. The Dr. Soetomo General Hospital's ethical committee approved this study (ethical approval certificate number: 0522/KEPK/ XI/2022).

Participants

The inclusion criteria were: 1) untrained healthy men aged 18–40 years; 2) normal musculoskeletal function (strength, sensation, and normal joint range of motion); 3) low or moderate physical activity level from the International Physical Activity Questionnaire-Short Form (IPAQ-SF) questionnaire; 4) agreement to be the study subject and consent to participate in the study by signing an informed consent form. Exclusion criteria were: 1) resistance training for at least six months prior to the experiment's start; 2) exercise contraindications, such as a history of lower limb injuries, fractures, or surgeries; and 3) NMES contraindications, such as pacemaker use or an allergy to stimulation electrodes. The following are the drop-out criteria: 1) subjects stopped participating in the programs and refused to go on; 2) subjects were unable to finish the training in accordance with the study protocol (missing two training sessions in a row); 3) subjects complained of joint or muscle pain (Visual Analogue Scale > 2) during active movements (without load); or there were symptoms of inflammation that suddenly appeared during exercise and delayed-onset muscle soreness (DOMS) in the lower limbs, so that the subject could not perform the exercise for two consecutive times. 4) The research subject was unable to proceed with the study due to an allergic reaction to the electric pad that occurred during the study.

Twenty-two participants were recruited using the consecutive sampling technique. The sample size in this study is based on the formula:

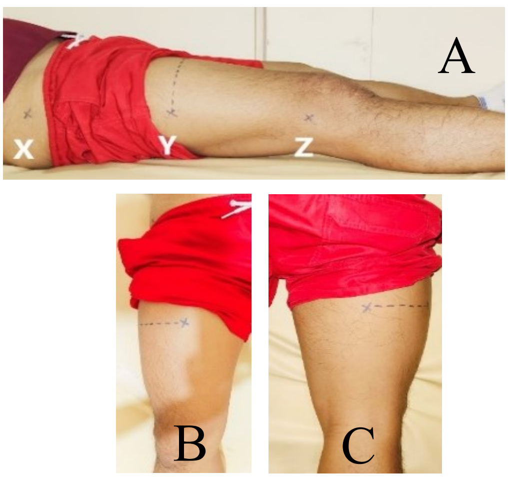
$$n_1=n_2=2\left[\frac{(Z\alpha+Z\beta)S}{(X1-X2)}\right]^2$$

With statistical calculations, the minimum sample size for each group is 8. With an anticipated dropout rate of 30%, the number of research subjects for each group is at least 11 people. So that the total number of subjects in this study is at least 22 people. Informed consent was obtained from all individual participants included in the study. Participants were divided into 2 groups: the modified hybrid training group (Hybrid) and the NMES Russian protocol group (NMES). Group division was done using simple randomization by drawing using a sealed envelope. We did triple-blind procedures which participants, researchers, and data analysts are unaware of their group assignment, throughout the study, we used coded labels (e.g., 'Group A' and 'Group B'). We also avoided discussing hypotheses or expected outcomes to prevent influencing behavior or expectations. Leg dominance was determined using the ball kick test (van Melick et al., 2017).

Pre and Post Intervention Examination

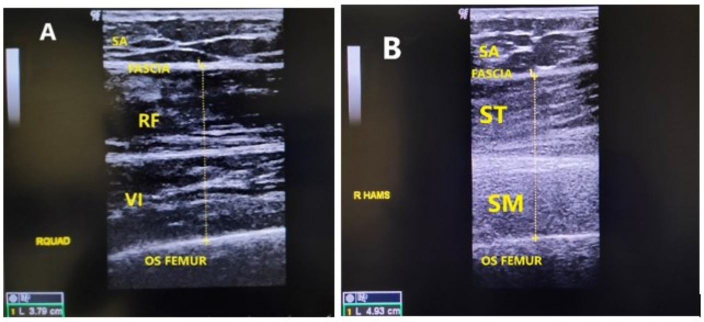
Prior to the procedure, all subjects had their quadriceps and hamstring muscle thickness measured using B-mode ultrasound (Logiq P6) at the anterior and posterior [50% of thigh length (TL)] aspects of the dominant and non-dominant thighs (Pringga et al., 2021). Figure 1A shows the measurement points of the ultrasound (USG) examination of the quadriceps and hamstring muscles. Thigh length was measured using anatomical landmarks (the distance between the most proximal side of the greater trochanter [point X] and the most distal side of the lateral femoral condyle [point Z], identified by palpation), and the measurement sites were marked with a marker pen. From point X to point Z, a midpoint (point Y) was taken. Point Z is drawn anteriorly at the middle of the thigh (Fig. 1B) as landmark of the quadricep. Point Z is drawn posteriorly at the middle of the thigh (Fig 1C) as landmark of the hamstring.

Figure 1. A: Lateral Thigh, B: Anterior Thigh, C: Posterior Thigh



All participants were instructed to lie down in a relaxed position. All images were taken in the longitudinal plane relative to the examined limb. Muscle thickness was defined as the distance between the bone-muscle interface and the adipose-muscle interface (Franchi et al., 2018). Measurements were taken from the most superficial side of the bright line, which represents the interface between the muscle and bone, to the most superficial side, which represents the interface between the muscle and subcutaneous adipose tissue. The anterior and posterior thigh USG longitudinal views are shown in Figure 2. For data analysis, three images from each location were assessed, and the average value of each location was used (Abe et al., 2014). Muscle thickness measurement was conducted by two physical medicine and rehabilitation specialists certified in ultrasonographic examination.

Figure 2. A: Anterior thigh. Legend: SA, Subcutaneous Adipose; RF, Rectus Femoris muscle; VI, Vastus Intermedius muscle; RQUAD, Right Quadricep; dash line, Quadricep muscle thickness. B: Posterior thigh. Legend: SA, Subcutaneous Adipose. ST, Semitendinosus muscle; SM, Semimembranosus muscle; R HAMS, Right Hamstring; dash line, Hamstring muscle thickness



Intervention

Hybrid group. With a hip flexion angle of 900 and a knee flexion angle of 1000, the patient was seated upright and leaning as comfortably as possible. Stimulation electrodes were placed on the hamstring motor belly at a minimum distance of 2 inches. Voluntary knee extension contractions were performed when the subject felt electrical stimulation in the hamstring (Priyantono, et al., 2023).

NMES group. The patient lay as relaxed as possible with a hip flexion angle of 10-200 and a knee 10-200. The knee was supported using a small pillow, and the distal part of the cruris was secured with a strap to ensure isometric contraction. Stimulation electrodes were placed on the quadriceps belly motor at a minimum distance of 2 inches (Priyantono, et al., 2023).

In both groups, each session consisted of 10 sets with 10 repetitions, with a 1-minute rest between sets. Each group got 12 sessions that were carried out in 3 sessions per week for 4 weeks. Intervention was done on both legs. Both groups used the same stimulation parameters: Russian stimulation with a frequency of 5000 Hz modulated at a frequency of 20 Hz (2.4 ms on, 47.6 ms off). The intensity was 80% maximal comfortable intensity, which will be adjusted at the beginning of each exercise. This level of intensity has been shown to effectively enhance muscle strength and mass without inducing pain (Tajima et al., 2021). We used the electrical stimulation Myomed 632 (Enraf-Nonius) as an electrical stimulator. After 4 weeks (12 times of exercise), muscle thickness of the quadriceps and hamstrings was remeasured.

Both approaches focused on the quadriceps muscle. In the Hybrid group, participants extended their knees while their hamstrings were electrically stimulated. Conversely, in the NMES group, electrical stimulation was applied directly to the quadriceps. The study aimed to compare the impact of direct electrical stimulation of the quadriceps (NMES group) with voluntary quadriceps contraction resisted by electrically stimulated hamstrings (Hybrid group). It also investigated whether the NMES group, which did not involve hamstring stimulation, experienced any indirect effects on hamstring muscle endurance compared to the direct hamstring stimulation in the Hybrid group.

Statistical Analyses

The statistical package for social sciences (SPSS version 26.0) was used to conduct the statistical analyses. The characteristics of the mean and standard deviation were ascertained through the presentation of descriptive data. The Monte Carlo test was used to perform the data normalcy test. Normal distribution if p > 0.05. The homogeneity test was analyzed with the Levene's test. The data variance is homogeneous if p > 0.05. We compared the two groups' pre- and post-intervention muscle thickness differences. The paired t-test was used if the data are normally distributed. In the event that the data exhibit an non-normal distribution, we used the Wilcoxon test. We also used the independent sample t test to compare the group differences (delta) in muscle thickness if the data is normally distributed. Conversely, if the distribution is not normal, the Mann-Whitney test was used. The differences were considered statistically significant at p < 0.05. Cohen's d effect size was performed to compare the effect of increasing muscle thickness between the groups. The interpretation of the effect size is seen from the value of d as follows: small (d = 0.2), medium (d = 0.5), large (d ≥ 0.8).

Results

The training sessions and study protocol were completed in twenty-two subjects. Homogeneity and normality tests showed no significant differences between groups in age, height, weight, BMI, or pre-intervention muscle thickness (Table 1), ensuring these factors did not influence the study results.

Table 1. Characteristics of subjects at baseline (Mean ± SD)

Characteristics		Hybrid group (n = 11)	p-value [a] (Normality)	NMES group (n = 11)	p-value [a] (Normality)	p-value [b] (Homogenity)
Age (year)		31.6 ± 2.77	0.783	32 ± 3.92	0.477	0.229
Bodyweight (kg)		74.63 ± 8.57	0.717	75 ± 11.33	0.995	0.329
Height (m)		1.71 ± 0.50	0.570	1.71 ± 0.57	0.684	0.98
Body mass index (kg/m2)		25.50 ± 3.36	0.605	25.72 ± 3.32	0.997	0.76
IPAQ-SF		Low : 2 (18,2%)		Low : 6 (54,6%)		
		Moderate : 9 (81,8%)		Moderate : 5 (45, 5%)		
Dominant leg		Right: (100%)		Right: (100%)		
Muscle Thickness Quadricep pre	Dom	42.97 ± 6.06	0.368	40.73 ± 3.87	0.956	0.51
	Non Dom	42.68 ± 6.88	0.142	41.50 ± 5.51	0.451	0.50
Muscle Thickness Hamstring pre	Dom	53.67 ± 7.20	0.793	50.81 ± 8.53	0.968	0.67
	Non Dom	53.27 ± 5.03	0.976	52.07 ± 8.31	0.717	0.35

[a] = Monte Carlo test
[b] = Levene test

Table 2 summarizes the changes in quadriceps and hamstring muscle thickness (Δ muscle thickness) before and after the intervention in the hybrid and NMES groups. Data were normally distributed, as confirmed by the Monte Carlo exact test, allowing for parametric paired and independent t-tests.
In the hybrid group, after four weeks significant increases were observed in the thickness of the dominant quadriceps (p = 0.019, d = 0.838), non-dominant quadriceps (p = 0.007, d = 1.014), dominant hamstring (p = 0.013, d = 0.912), and non-dominant hamstring (p = 0.002, d = 1.287). In the NMES group, significant increases were found in the dominant (p = 0.011, d = 0.946) and non-dominant quadriceps (p = 0.002, d = 1.291), but not in the dominant (p = 0.269) or non-dominant hamstring (p = 0.092).

Table 2. Comparison of Quadriceps and Hamstrings Muscle Thickness Before and After Intervention

Variable	Leg Side	Hybrid Group			NMES Group			Comparison between 2 groups	
		Δ muscle thickness[a]	P value [b]	Effect size [d]	Δ muscle thickness[a]	P value [b]	Effect size [d]	P value [c]	Effect size [d]
Quadricep Femoris	Dom	2,51 ± 3,0	0.019	0,838	2,38 ± 2.51	0.011	0.946	0.909	0.049
	Non Dom	2,62 ± 2,59	0.007	1,014	2.40 ± 1.87	0.002	1.291	0.826	0.095
Hamstring	Dom	3.09 ± 3,39	0.013	0,912	0.81 ± 2.23	0.269	0.353	0.079	0.789
	Non Dom	3,2 ± 2,5	0,002	1,287	0.83 ± 0.92	0.092	0.563	0.004	1.396

[a] = Increased muscle thickness in milimeter (Mean ± SD)
[b] = paired sample t test, [c] = independent t-test,

[d] = Cohen's d Effect size

Comparison between groups revealed no significant differences in quadriceps thickness changes for either leg (dominant: p = 0.909; non-dominant: p = 0.826) or in the dominant hamstring (p = 0.079). However, a significant difference was noted in the non-dominant hamstring thickness (p = 0.004) with a large effect size (d = 1.396).

Discussion

This study demonstrated significant muscle hypertrophy in both groups, with particularly notable improvements in the hybrid group. In the hybrid group, significant increases were observed in quadriceps and hamstring muscle thickness on both sides of the leg after 4 weeks of intervention, accompanied by large effect sizes across all muscles. On average, muscle thickness increased by 5.8% in the dominant quadriceps, 6.32% in the non-dominant quadriceps, 5.95% in the dominant hamstrings, and 6.31% in the non-dominant hamstrings. These results align with Ito et al., (2004) who reported a 5.3% increase in the knee flexor-extensor cross-sectional area following hybrid training performed three times weekly for 4 weeks in healthy men. Similarly, Takano et al., (2010) found increases in muscle cross-sectional area of 9% in the hybrid training group and 14% in the weight machine training group after training twice weekly for 2 weeks in the elderly.

The increase in quadriceps thickness reflects physiological adaptations, including muscle hypertrophy and hyperplasia, which typically emerge after 3–4 weeks of moderate- to high-intensity strengthening exercises (Fisher et al., 2013). The increase in hamstring thickness results from the contraction of muscle fibers stimulated by electrical impulses. The concurrent increases in quadriceps and hamstring muscle thickness highlight a key advantage of the HTS, which integrates voluntary quadriceps contraction with antagonist hamstring contraction via electrical stimulation, producing simultaneous activation of both muscle groups (Rabe et al., 2018).

In the NMES group, there was a significant increase in quadriceps muscle thickness on both sides of the leg, accompanied by a large effect size. However, no significant increase was observed in hamstring muscle thickness. On average, quadriceps thickness increased by 5.8% in both the dominant and non-dominant legs, while the increases in hamstring thickness were 1.6% and 1.07% for the dominant and non-dominant sides, respectively. These findings are consistent with Modesto et al. (2020), who reported an 8.7 ± 3.8% increase in Vastus Lateralis muscle thickness after NMES Russian Current was applied three times weekly for six weeks in soccer players (Modesto et al., 2020). The increase in quadriceps thickness is attributed to the stimulation of type 2 muscle fibers, which are capable of producing strong and rapid contractions but fatigue quickly. The absence of a significant increase in hamstring thickness is likely due to the lack of direct electrical stimulation or other targeted interventions for this muscle group (Cameron, 2018).

This study observed weak statistical evidence for differences in quadriceps muscle thickness changes on either side or in the dominant hamstring between the hybrid and NMES groups. However, there was strong statistical evidence for differences in non-dominant hamstring muscle thickness (p = 0.004) with a large effect size (d = 1.396), highlighting a meaningful impact of both interventions on non-dominant hamstring hypertrophy. The hybrid-modified strengthening exercise demonstrated a notable advantage by inducing simultaneous hypertrophy of both agonist and antagonist muscles, whereas the NMES group targeted only the quadriceps.

The greater increase in non-dominant hamstring muscle thickness (p = 0.004) compared to the dominant side (p = 0.079) may be attributed to enhanced neural adaptation in the non-dominant limb. This phenomenon is likely due to lower baseline motor unit activation in the non-dominant limb, often associated with reduced loading in daily activities, making it more responsive to training stimuli (Fisher et al., 2013). Over the 4-week intervention, neural adaptations in the non-dominant limb, such as improved motor unit recruitment and firing rates, likely contributed to greater hypertrophy and strength gains (Modesto et al., 2020). In contrast, the dominant limb, already exhibiting higher motor unit activation due to habitual use, showed no strong statistical evidence for differences in muscle thickness changes between the hybrid and NMES groups, potentially due to a ceiling effect in adaptation.

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phenomenon is likely due to lower baseline motor unit activation in the non-dominant limb, often associated with reduced loading in daily activities, making it more responsive to training stimuli (Bogdanis, 2012, Wiriawan et al., 2024). Over the 4-week intervention, neural adaptations in the non-dominant limb, such as improved motor unit recruitment and firing rates, likely contributed to greater hypertrophy and strength gains (Del Vecchio et al., 2019). In contrast, the dominant limb, already exhibiting higher motor unit activation due to habitual use, showed no strong statistical evidence for differences in muscle thickness changes between the hybrid and NMES groups, potentially due to a ceiling effect in adaptation.

This study focused on healthy, untrained males, which limits the generalizability of the findings to other populations. The choice to study only male subjects was based on the hypothesis that they experience greater muscle hypertrophy after resistance training, potentially due to differences in gene expression and higher testosterone levels compared to females, making the progression of hypertrophy more apparent in males (Refalo et al., 2024). We choose healthy people for safety and convenience reasons because our study was the first to use a modified hybrid training protocol outside its primary research center in Japan, and no data on its effectiveness or safety were available in our center.

The 4-week duration of the hybrid strengthening and Russian NMES stimulation protocols in this study was shorter than the 4–12 weeks typically required to observe muscle hypertrophy (Kisner et al., 2018). This timeframe was chosen based on previous research, such as Ito et al. (2004), which reported a 5.3% average increase in muscle mass following hybrid training three times weekly for 4 weeks in healthy males.) (Ito et al., 2004). Additionally, both MHT and NMES carry a risk of muscle fatigue due to their tendency to preferentially activate fast-twitch muscle fibers. These fibers produce stronger and faster contractions but fatigue quickly and are prone to weakness or atrophy if underutilized. Electrically induced contractions are also more exhausting than physiological contractions (Cameron, 2018), motivating the decision to limit the study duration to minimize potential side effects.

To reduce biases that might influence the outcomes of this study, we controlled for physical activity by monitoring participants' daily activities and prohibiting strength training during the study period. Participants with resistance training experience within the prior six months were excluded. We minimized nutritional bias by ensuring participants avoided muscle-building diets or supplements.

Conclusions

MHT and the NMES Russian protocol were shown to increase Quadriceps Femoris and Hamstring hypertrophy after 4 weeks. In addition, MHT was shown to increase non-dominant side Hamstring muscle hypertrophy greater than the NMES Russian protocol. This study demonstrated the safety and effectiveness of the methods in increasing muscle mass in healthy men. Future research could extend this approach to other groups, such as women, older adults, and patients. To provide an alternative for those with limited time or access to strength training facilities, further research with longer intervention periods is needed to better evaluate muscle hypertrophy and compare its effectiveness to resistance training.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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