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Hemodynamics and Systemic Fibrinolytic Activity: A Randomized Clinical Pilot Study

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Abstract

Objective

Muscular physical exercise is a stimulus to circulatory development. But it is not always possible. In this context, there was the development of intermittent pneumatic compression therapy and, more recently, neuromuscular electrical stimulation.

Methods

A clinical, interventional, prospective, single-center, randomized, single-blind study was carried out to evaluate the effect of neuromuscular electrical stimulation on lower limb hemodynamics and systemic fibrinolytic activity in 10 healthy subjects. The study protocol was approved by the Research Ethics Committee of the Botucatu Medical School, UNESP (CAAE: 30680120.0.0000.5411).

Results

The flow volume variation was significant, in the joint analysis of the limbs, at the popliteal vein. The diameter of the vessels proved to be the most sensitive to neuromuscular electrical stimulation in the joint analysis of the limbs. Flow velocities varied statistically significantly only in the femoral vessels. In the popliteal vessels, there was a variation in flow increment, but it was not statistically significant. There was no statistically significant change in the plasminogen activator inhibitor. For plasminogen activator, there was numerical variation, with continuous increment, but this did not represent a statistically significant change ($p > 0.05$). There was no adverse event and all subjects had the lowest tolerance score (no feeling of discomfort).

Conclusions

NMES significantly improved popliteal and femoral hemodynamics. Although numerical increases in plasminogen activator were observed, they did not reach statistical significance ($p > 0.05$). The protocol proved to be safe and well-tolerated.

Trial Registration:

Brazilian Registry of Clinical Trials (ReBEC) **RBR-6gs4bdh**. UTN: **U1111-1301-5055**. Registered on January 29, 2024. URL: https://ensaiosclinicos.gov.br/rg/RBR-6gs4bdh

1. INTRODUCTION

The prevalence of peripheral vascular diseases is steadily increasing, primarily driven by the aging global population and the concomitant rise in cardiovascular risk factors.[1] The three most clinically significant conditions in this domain are peripheral arterial disease (PAD), chronic venous insufficiency (CVI), and venous thromboembolism (VTE).

The therapeutic management of these patients involves two primary pillars: symptomatic relief and the mitigation of systemic cardiovascular risks. Preventive strategies are paramount, encompassing optimized pharmacological therapy alongside essential non-pharmacological measures.[2]

Physical exercise, particularly muscle-strengthening and aerobic activities, serves as a potent stimulus for circulatory adaptation. As demonstrated by Lauret et al. (2014)[3], the benefits of structured exercise programs are substantial, including improvements in pain-free walking distance, functional status, and overall quality of life, while simultaneously reducing cardiovascular mortality.[4] In the venous system, Padberg et al. (2004) evidenced that calf muscle pump function and muscular strength significantly improve after structured exercise intervention.[2]

Despite these proven benefits, poor adherence remains a major obstacle in clinical practice, particularly among PAD patients. Rehabilitation programs are often underutilized and require active promotion to be effective.[4] Furthermore, conventional walking-based exercise is not always feasible for patients with severe comorbidities or physical limitations. A Cochrane review highlighted that while supervised walking remains the gold standard, alternative forms of exercise—such as cycling or strength training—may serve as viable substitutes when walking is contraindicated or impossible.[3]

Beyond its well-documented effects on arterial and venous hemodynamics, physical exercise also modulates the fibrinolytic system, which plays a critical role in vascular homeostasis.[5] However, the potential for non-voluntary muscle activation, such as neuromuscular electrical stimulation (NMES), to replicate these hemodynamic and fibrinolytic benefits remains an area requiring further investigation, particularly in the context of patients unable to perform traditional exercise.

1.1 NEUROMUSCULAR ELECTRICAL STIMULATION (NMES)

First-line therapy for patients with intermittent claudication typically includes supervised exercise programs aimed at improving walking distances and functional capacity. However, conventional exercise presents significant barriers, including low patient compliance, physical limitations, and contraindications in specific clinical conditions.[6]

In this scenario, neuromuscular electrical stimulation (NMES) has emerged as a promising therapeutic alternative. NMES involves the application of transcutaneous electrical currents to trigger involuntary muscle contractions, thereby activating the calf muscle pump—often referred to as the "peripheral heart." This mechanism potentially replicates the hemodynamic benefits of active exercise, such as increased venous return and improved arterial inflow, without requiring voluntary physical exertion or joint loading.

Consequently, NMES may offer a viable circulatory stimulus for patients who are otherwise unable to participate in traditional rehabilitation programs.

1.1.2 Percutaneous electrical muscle stimulation (PEMS)

In percutaneous electrical muscle stimulation (PEMS), the musculature is stimulated via transcutaneous electrodes placed directly over the muscle bellies, aiming for localized activation rather than generalized neurological stimulation.[7]

Early evidence supporting this modality emerged in the 1980s, when Currier et al. (1986) demonstrated the physiological benefits of PEMS on circulatory parameters.[8] Subsequent research in 1997 further established that continuous PEMS-induced contractions in the lower limbs (thigh and leg) effectively activate the physiological muscle pump. This mechanism was shown to enhance venous circulation and reduce blood stasis during surgical procedures, suggesting that PEMS could offer superior protection against deep vein thrombosis (DVT) and pulmonary embolism (PE) compared to conventional intermittent pneumatic compression (IPC).[9]

More recently, a 2016 study evidenced that PEMS significantly improved maximal walking capacity in claudicant patients with type 2 diabetes.[6] These findings support the use of PEMS as a viable therapeutic approach for patients with impaired exercise tolerance or as a potent adjunctive treatment modality. Current evidence indicates that PEMS significantly enhances both local and systemic hemodynamic parameters.[7] However, the clinical application of this technology remains partially limited by patient comfort and significant variability in individual physiological responses.[7]

1.1.3 NEUROMUSCULAR ELECTRICAL STIMULATION (NMES)

Another effective modality to enhance arterial and venous flow is Neuromuscular Electrical Stimulation (NMES), which targets the motor nerves of the lower limbs.[10] This method involves the application of transcutaneous electrical pulses through surface electrodes to trigger neural action potentials, subsequently inducing coordinated muscle contractions.[11]

A significant advantage of NMES over direct percutaneous muscle stimulation (PEMS) is the requirement of a substantially lower electrical current to achieve therapeutic muscle activation. This characteristic results in improved patient comfort and a lower incidence of adverse events, such as skin burns.[12] Furthermore, a 2014 study demonstrated that specific NMES protocols were more effective than intermittent pneumatic compression (IPC) in increasing venous and arterial flow, as well as microcirculatory blood velocity.[10]

1.1.3.1 Application in Arterial Disease

In the arterial territory, NMES serves as a form of "involuntary exercise." By replicating the metabolic and mechanical demands of physical activity, it may promote the development of collateral circulation and improve distal perfusion in patients with peripheral obstructive atherosclerotic disease (PAD).[13]

1.1.3.2 Application in Venous Disease

In the venous territory, the primary clinical applications of NMES include deep vein thrombosis (DVT) prophylaxis, the treatment of chronic venous ulcers, and the management of post-thrombotic syndrome.[14] By activating the calf muscle pump, NMES effectively reduces venous stasis and enhances ambulatory venous pressure, addressing the core pathophysiology of chronic venous insufficiency (CVI).

Aqui está a revisão técnica dessas subseções, refinando a linguagem científica e garantindo que os mecanismos fisiopatológicos e as evidências de estudos anteriores (como os de Broderick e Hajibandeh) sustentem a relevância do seu trabalho.

1.1.3.2.1 Application in VTE Prophylaxis

Neuromuscular Electrical Stimulation (NMES) serves as a potent tool for reducing the incidence of venous thromboembolism (VTE). By inducing rhythmic muscle contractions, it significantly accelerates venous flow velocity and volume, thereby mitigating the primary risk factor for thrombus formation: venous stasis.[14]

1.1.3.2.2 Application in Chronic Venous Insufficiency (CVI)

The management of severe CVI, whether of primary origin or secondary to post-thrombotic syndrome, necessitates addressing venous hypertension—the central pathophysiological driver of the disease and its associated venous ulcers.[15]

Evidence from Moloney et al. (2006) demonstrated that combining NMES with compression bandages in patients with CVI and active ulceration led to a significant increase in venous outflow. These findings support the hypothesis that NMES may facilitate or accelerate wound healing by improving regional microcirculation.[16]

Furthermore, Broderick et al. (2014) established that NMES produces significantly larger ejected volumes compared to intermittent pneumatic compression (IPC)—reaching 3.0 times greater volumes in the foot and 1.7 times greater in the calf. This superior performance is attributed to the more effective emptying of the soleus veins and sinusoids, which is clinically critical as these are the predominant sites for deep vein thrombosis (DVT) initiation.[17]

Unlike IPC, NMES devices are silent, portable, and less restrictive, overcoming common barriers to patient adherence. Consequently, NMES shows great potential for both hospital and home-based prophylaxis in high-risk populations.[17] A systematic review by Hajibandeh et al. (2015) further confirmed that NMES significantly enhances venous flow and is exceptionally well-tolerated by patients.[18]

1.1.3.3 Modulation of the Fibrinolytic System

Patients with lower limb vascular diseases are predisposed to a prothrombotic state. Investigating the impact of electrical stimulation on fibrinolytic activity is therefore of high clinical interest. One study evaluated whether common peroneal nerve stimulation could promote systemic fibrinolysis by comparing an active limb (receiving NMES) with a contralateral control limb. The study concluded that such stimulation can enhance fibrinolytic activity by decreasing plasma levels of type-1 plasminogen activator inhibitor (PAI-1) in patients with both arterial and venous diseases of the lower limbs.[19]

2. OBJECTIVES

2.1 Primary and Secondary Objectives

The primary objectives of this pilot study were:

a) To evaluate the acute effects of a specific neuromuscular electrical stimulation (NMES) protocol on arterial and venous hemodynamics (flow volume and velocity) in the lower limbs of healthy individuals.

b) To investigate the impact of NMES on the systemic fibrinolytic system by measuring plasma levels of plasminogen activator and its inhibitor (PAI-1).

c) To assess whether the localized application of NMES induces measurable systemic circulatory and fibrinolytic changes.

The secondary objectives were:

a) To evaluate the safety of the NMES protocol and the participant's tolerability/comfort during the procedure using a standardized discomfort scale.

3. MATERIALS AND METHODS

3.1 Study Design and Ethical Considerations

A clinical, interventional, prospective, single-center, randomized, single-blind pilot study was conducted to evaluate the effects of Neuromuscular Electrical Stimulation (NMES) on lower limb hemodynamics and systemic fibrinolytic activity.

The study protocol and informed consent forms were reviewed and approved by the Research Ethics Committee of the Botucatu Medical School, UNESP, Brazil (CAAE: 30680120.0.0000.5411; Approval Protocol No. 4.105.940). All procedures were performed in strict accordance with the relevant guidelines and regulations, including the Declaration of Helsinki and the CONSORT guidelines for reporting randomized trials. All participants provided written informed consent prior to their inclusion in the study.

The trial was registered at the Brazilian Clinical Trials Registry (ReBec) under the identifier RBR-6gs4bdh (UTRN: U1111-1301-5055). Although the study was registered on January 29, 2024, recruitment and data collection followed the approved institutional protocol.

3.2 Participants

Ten (10) healthy subjects were recruited for this pilot investigation. The use of a healthy cohort served as a physiological "proof of concept" to establish baseline hemodynamic and fibrinolytic responses to the NMES protocol in the absence of confounding vascular pathologies.

3.3 Elegibility criteria

3.3.1 Participant Recruitment and Screening

Ten healthy volunteers were invited to participate in the study. Screening was conducted at the Vascular Laboratory of the Hospital das Clínicas, Botucatu Medical School (UNESP), Brazil. The screening process included a detailed clinical anamnesis and physical examination to verify eligibility. Individuals aged 18 to 40 years were selected to minimize the influence of age-related endothelial dysfunction and altered vascular reactivity on the results.[20'21]

3.3.2 Inclusion Criteria

Participants were included if they met all the following requirements:

1. Age between 18 and 40 years;[21]
2. Provision of written informed consent;
3. Body Mass Index (BMI) between 18 and 34 kg/m².[22]

3.3.3 Exclusion Criteria

To ensure the stability of vascular reactivity and fibrinolytic parameters, the following exclusion criteria were applied:

- Pharmacological and Substance Use: Use of illicit drugs in the 12 months prior to the study; use of medications with vascular effects (e.g., sumatriptan, ergot derivatives, isometheptene), analgesics (dipyrone, paracetamol), or non-steroidal anti-inflammatory drugs (NSAIDs) within 24 hours of the intervention. Active or former smokers (within the last 12 months) were also excluded.
- Dietary Restrictions (24-hour washout): Consumption of foods or substances known to affect vascular reactivity, including coffee,[23'24] folic acid, vitamin C, high-dose fish oil,[25] vitamin supplements,[26] large quantities of specific fruits/vegetables (cranberry, grapefruit, beetroot, black currant),[27'28] flavonoids (wine, tea, cocoa, chocolate, hazelnuts),[29'30] natural grape or orange juice,[31] cheese,[32] probiotics,[33] capsaicin (peppers),[34'35] energy drinks,[36] and alcohol (beer).[37]

- Medical and Surgical History: Any significant illness in the 4 weeks prior to the study; history of peripheral arterial disease (Rutherford 1–6) or an ankle-brachial index (ABI) < 0.9;[38] history of chronic venous insufficiency (CEAP 2–6);[39] history of venous thromboembolism (VTE) or lower limb ulcers. Participants with a history of lower limb fractures, metallic implants, or recent surgery (abdominal or gynecological) within the last 3 months were also excluded.
- Physical and Device-related Contraindications: Pregnancy or lactation; use of hormonal contraceptives (including IUDs) within the last 3 months; presence of pacemakers or implantable cardioverter-defibrillators; use of neuromodulatory devices or participation in other clinical trials within 4 weeks.
- Local Conditions: Active infection at the electrode application site or lower limb trauma that could impede correct common peroneal nerve stimulation.

3.4 Experimental protocol

3.4.1 Study Timeline and Assessments

The experimental procedure was divided into six distinct stages:

- Moment 0 (M0): Initial positioning and 30-minute acclimatization/rest period.
- Moment 1 (M1): Baseline assessments (pre-stimulation).
- Moment 2 (M2): Active NMES intervention (20 minutes).
- Moment 3 (M3): Assessment immediately following NMES.
- Moment 4 (M4): Assessment 120 minutes post-intervention.
- Moment 5 (M5): Final assessment 240 minutes post-intervention.

Participants remained in a controlled environment (20–25 °C, humidity > 20%) in a seated position with feet elevated to hip level (allowing for knee flexion and external hip rotation) throughout the 4-hour monitoring period to ensure hemodynamic stability.

3.4.2 Hemodynamic and Biochemical Measurements

At M1, M3, M4, and M5, the following assessments were performed:

1. Duplex Ultrasound (DUS): Evaluated using a Vivid 9 system (GE Healthcare, Chicago, IL, USA) by a single blinded examiner. Bilateral measurements of the femoral and popliteal vessels included:
    - Peak Systolic Velocity (PSV): Measured in cm/s.
    - Vessel Diameter: Anteroposterior measurement in mm.
    - Flow Volume: Calculated automatically by the software in mL/min.
2. Laboratory Analysis: Venous blood samples were collected to quantify systemic fibrinolytic activity:
    - Plasma levels of tissue plasminogen activator (tPA).

- Plasma levels of plasminogen activator inhibitor type-1 (PAI-1).

3.4.3 NMES Intervention and Parameters

During M2, NMES was administered using the NEURODYN II device. Surface electrodes were placed bilaterally over the posterior and proximal aspects of the leg, targeting the common peroneal nerve to induce isometric muscle contractions. Although electrodes were placed on both limbs to maintain participant blinding, only the randomized "active" limb received the electrical stimulus.

The Functional Electrical Stimulation (FES) current was configured with the following standardized parameters:

- Waveform: Bipolar rectangular.
- Pulse width: 250 μs.
- Frequency: 50 Hz.
- Duty Cycle: 1s rise, 1s sustain, 1s fall.
- Duration: 20 minutes.

3.4.4 Safety, Tolerability, and Discontinuation

Tolerability was assessed using a 5-point Likert scale: 1 (no discomfort); 2 (minimal discomfort); 3 (slight discomfort); 4 (moderate discomfort); and 5 (severe discomfort). Scores of 4 or 5 were established as criteria for immediate study discontinuation. Other discontinuation criteria included withdrawal of consent, adverse events, or non-compliance with the resting position protocol.

3.5 Study outcomes and randomization

3.5.1 Primary Outcomes

The primary outcomes of this study focused on the hemodynamic and biochemical responses to NMES:

1. Local and Systemic Hemodynamics: Evaluation of NMES-induced changes in Peak Systolic Velocity (PSV), vessel diameter, and flow volume in the femoral and popliteal vessels. Measurements were taken bilaterally to assess both local effects (stimulated limb) and potential systemic responses (contralateral non-stimulated limb).
2. Fibrinolytic Activity: Quantification of systemic fibrinolytic modulation through plasma concentrations of tissue plasminogen activator (tPA) and plasminogen activator inhibitor type-1 (PAI-1).

3.5.2 Secondary Outcomes

The secondary outcomes assessed the clinical feasibility of the protocol:

1. Safety: Incidence of any expected or unexpected adverse events throughout the 4-hour experimental period.
2. Tolerability: Assessment of participant comfort using a standardized 5-point Likert scale.

3.5.3 Randomization and Allocation

To determine which lower limb (right or left) would receive active stimulation, a simple 1:1 randomization was performed. The allocation sequence was generated using a specialized digital randomizer (https://www.randomizer.org/), ensuring an unbiased distribution across the cohort.[40]

3.5.4 Adverse Events and Risk Management

Adverse events were categorized as follows:

- Expected Risks: Potential localized skin irritation or minor thermal injury (burns) under the electrode interface, as documented in NMES literature.[10'41]
- Unexpected Events: Any serious, life-threatening, or previously unidentified health issue associated with the device or protocol.

Safety monitoring was conducted continuously during the 20-minute intervention (M2) and throughout the subsequent 240-minute follow-up period.

3.6 Statistical analisys

Statistical analysis was conducted using a non-parametric approach, as the data distributions were assessed for normality and exhibited non-Gaussian characteristics. The Friedman test was employed for the longitudinal analysis of hemodynamic and biochemical variables across the five experimental moments (M1, M3, M4, and M5), followed by the Bonferroni post-hoc correction (Sigel and Castellan method) to account for multiple comparisons and control the family-wise error rate.

For the comparison between the stimulated (active) and non-stimulated (contralateral/control) limbs at specific time points, the Wilcoxon signed-rank test for paired data was utilized. This dual-analysis approach allowed for the separate verification of localized NMES effects and potential systemic responses.

All statistical estimates were performed with a significance level (alpha) of 5% ($p < 0.05$). Data processing, statistical testing, and graphical representations were developed using R software with the RStudio interface (Version 4.1.1, RStudio, Inc.).

The final sample consisted of 10 healthy individuals. Outliers were identified during the exploratory data analysis but were maintained in the final dataset as they remained within physiologically plausible reference standards and did not represent measurement errors.

4. RESULTS

The sample consisted of 10 individuals with the following demographic characteristics: 5 men and 5 women; Age (mean 30.4 / median 31 years); Weight (mean 70.2 kg / median 71.15 kg); Height (mean 1.66 m / median 1.66 m); and BMI (mean 25.48 kg/m² / median 25.50 kg/m²).

The joint analysis of the moments to identify differences between the stimulated vessels and those that were not stimulated was only significant for the peak velocity of the popliteal vein. In the rest of the vessels and variables analyzed, this difference was not observed. All subjects gave the lowest score for discomfort (Score 1) and there were no adverse events, expected or not.

4.1 PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1)

There was no statistically significant change in the plasminogen activator inhibitor during the moments. There was only numerical variation, less than 2%, as seen in Table I and Supplemental Tables I and II.

4.2 TISSUE PLASMINOGEN ACTIVATOR (tPA)

There was numerical variation, with continuous increment, of up to almost 6% in M4, but this did not represent a statistically significant change, as seen in Table I and Supplemental Tables I and II.

4.3 FLOW VOLUME

The variation in flow volume was significant in the joint analysis of the limbs, as seen in Table II, at moment 4 for the popliteal vein, with a decrease of 23.94%, from 12.135 ml/min to 9.23 ml/min, while the interquartile median (IQR) had a reduction of 40.22%, going from 11.98 ml/min to 7.16 ml/min.

4.4 VESSEL DIAMETER

Vessel diameters proved to be the most sensitive to NMES in the joint analysis of limbs, as seen in Table III.

- 4.4.1 Diameter of the femoral artery: The variation for M3 and M5 were significant. The median and IQR variation were, respectively: 11.03% (0.725-0.805mm) and 34.61% (0.13-0.175mm) for M3; and 10.35% (0.725-0.8mm) and 44.61% (0.175-0.188mm) for M5 (Supplemental Fig. 1).
- 4.4.2 Diameter of the femoral vein: The femoral vein diameter changed significantly at all times. The median and IQR varied, respectively: 38.28% (0.64-0.885mm) and 55.17% (0.29-0.45mm) for M3; 32.03% (0.64-0.845mm) and 36.89% (0.29-0.397mm) for M4; 39.06% (0.64-0.89mm) and 88.62% (0.29-0.547mm) for M5 (Supplemental Fig. 2).
- 4.4.3 Diameter of the popliteal artery: Only M3 was significant. The variation of the median and IQR diameter was 10.19% (0.54-0.595mm) and 6.89% (0.145-0.155mm) (Supplemental Fig. 3).
- 4.4.4 Diameter of the popliteal vein: The popliteal vein had the median diameter significantly increased at M3 and M4: 31% (0.5-0.655mm) and 43% (0.5-0.715mm), respectively (Supplemental Fig. 4).

4.5 FLOW VELOCITY

Flow velocities varied statistically significantly only in the femoral vessels, as seen in Table IV. In the popliteal vessels, there was a variation in flow increment, but it was not statistically significant (Supplemental Figs. 5 and 6).

- 4.5.1 Femoral artery Peak Systolic Velocity (PSV): The femoral artery showed significant increases in PSV at M4 and M5. The median and IQR varied, respectively: 31.39% (287.635–377.92 cm/s) and 51.71% (155.247–235.52 cm/s) for M4; and 46.09% (287.635–420.20 cm/s) and 10.09% (155.24–170.91 cm/s) for M5 (Supplemental Fig. 7).
- 4.5.2 Femoral vein Peak Velocity: The median femoral vein peak velocity increased significantly, ranging from 22.22% (239.99–385.125 cm/s) at M4 and 33.63% (239.99–421.07 cm/s) at M5 (Supplemental Fig. 8).

5. DISCUSSION

Neuromuscular electrical stimulation (NMES) has been an object of medical interest for several decades. A search on *ClinicalTrials.gov* using the term "NMES" yields over three hundred results, spanning from orthopedic rehabilitation to treatments for dysphagia and diabetic complications. As electrical therapy continues to evolve, it remains a frontline candidate for developing new therapeutic modalities across various diseases.

This study sought to establish the acute effects of NMES, applied via the NEURODYN II device, on lower limb hemodynamics and systemic fibrinolytic activity in a cohort of 10 healthy individuals. Although participants were invited randomly, the application of strict inclusion and exclusion criteria meant that few individuals met full eligibility; the final participant was included approximately one month before the conclusion of the study period.

The rigorous screening process resulted in a balanced group of five men and five women with healthy lifestyles—some of whom were athletes—who successfully adhered to the experimental dietary restrictions. This dietary control, a factor often overlooked or unmentioned in similar studies, was implemented to minimize external variables that could interfere with vasomotricity, particularly flow-mediated dilation (FMD).

5.1 Rationale of Eligibility Criteria

Flow-mediated changes in arterial diameter are primarily driven by shear-stress-induced generation of endothelium-derived vasoactive mediators.[42] Bioactive compounds in various foods can interfere with FMD through mechanisms that are not yet fully elucidated. While some substances, such as specific fruit juices, may exert long-term effects (up to one month),[31] we opted for a 24-hour washout period for

the most common and known bioactive foods to minimize immediate interference with circulatory measurements.

5.2 RATIONALE FOR DEVICE CONFIGURATION

This study employed Functional Electrical Stimulation (FES), which utilizes low-frequency electrical currents to elicit specific, functional muscle contractions. FES is clinically indicated for neuromuscular facilitation, spasticity control, management of paretic or plegic muscles, and treating disuse atrophy.[43]

The selection of stimulation frequency is primarily dictated by the target muscle fiber types: 20–40 Hz is typically chosen for Type I (slow-twitch) fibers, while 60–80 Hz is required for Type II (fast-twitch) fibers.[44] Similarly, the pulse width (duration) is determined by the muscle volume to be recruited; large muscle groups (e.g., quadriceps) generally require longer pulses (200–400 µs), whereas smaller groups (e.g., wrist extensors) respond to shorter pulses (100–200 µs).[44]

To prevent premature muscle fatigue, appropriate "ON" (contraction) and "OFF" (rest) times must be established. The duration of these intervals is typically proportional to the patient's baseline muscle strength—lower strength requires longer rest periods.[44] While total stimulation time can vary between 20 and 60 minutes depending on the clinical objective,[45] evidence suggests that prolonged tetanic contractions do not provide additional venous flow benefits after the initial contraction, as the targeted veins are rapidly emptied. This physiological observation led to the adoption of balanced stimuli protocols, such as single pulses (e.g., 250 µs) delivered every second.[46]

These optimized parameters not only provide efficient blood pumping but also significantly enhance user tolerability and reduce metabolic demand, thereby preventing fatigue during prolonged use.[46] In this research, intensity was increased until visible muscle movement was achieved. This decision was supported by evidence demonstrating that NMES protocols reaching visible contraction can increase microcirculation by a factor of three compared to intermittent pneumatic compression (IPC). Even at sub-visible contraction intensities, NMES has been shown to increase blood flow by 23% more than IPC devices.[47]

Regarding frequency selection, experimental data indicates that reducing frequency (e.g., from 100 Hz to 25 Hz) can decrease muscle fatigue from 76% to 39%, whereas variations in pulse amplitude and duration appear to have a negligible impact on fatigue markers.[48]

5.3 EFFECTS ON HEMODYNAMICS

5.3.1 Vessel Diameter

Among all variables analyzed, vessel diameter proved to be the most sensitive to NMES. A consistent increase in diameter was observed in all analyzed vessels, with significant findings persisting at moments M4 and M5. Notably, these variations showed no statistical difference between the stimulated and non-stimulated (contralateral) limbs, which allowed for a joint analysis of the data and strongly

suggests a systemic effect of NMES. This vasodilation occurred even when flow volume variations were not significant, suggesting that the effect was not merely a passive dilation caused by increased flow. The continuous increase in the diameter of the popliteal arteries (with the highest median recorded at M5) suggests a prolonged residual effect of NMES that continues well after the stimulation period has ended.

5.3.2 Flow Velocities

Flow velocities varied without statistical difference between limbs over time, with the exception of the popliteal vein. Significant changes in peak velocities were primarily observed in the femoral vessels (both arterial PSV and venous peak velocity). While velocities in the popliteal vessels trended positively, they did not reach statistical significance. The clinical relevance of these increased velocities supports the application of NMES in therapies requiring enhanced circulatory flow, such as VTE prophylaxis and potential management of CVI and PAD.

5.3.3 Flow Volume

Flow volume variations were observed in both the stimulated and non-stimulated limbs, with no statistical difference between them. In the joint analysis, the popliteal vein showed statistically significant variations between M4 and M1, and between M5 and M4, further supporting a late or residual effect of the NMES protocol.

5.4 EFFECTS ON THE FIBRINOLYTIC SYSTEM

Laboratory analysis of systemic fibrinolytic activity (tPA and PAI-1) showed no statistically significant changes. However, tissue plasminogen activator (tPA) exhibited a continuous numerical increment, reaching an increase of nearly 6% at M5. Conversely, the plasminogen activator inhibitor (PAI-1) showed numerical variations close to zero. These findings suggest a subtle numerical trend toward increased fibrinolytic activity that may require larger cohorts or different stimulation durations to reach statistical significance.

5.5 SYSTEMIC EFFECTS AND BLINDING PERFORMANCE

The evaluation of the contralateral limb demonstrated that unilateral NMES induces variations in diameter and velocity similar to those in the stimulated limb, with no statistical difference in most parameters. This symmetry allowed for the pooled analysis of the data. The only exception was the popliteal vein, where the stimulated side presented a statistically superior performance in peak velocity distribution compared to the non-stimulated side. This distinction underscores the localized mechanical efficiency of the calf muscle pump activation.

5.6 SAFETY AND TOLERABILITY

The safety and tolerability of this NMES protocol were confirmed by the complete absence of adverse events. All subjects reported the lowest possible discomfort score (Score 1), which distinguishes this

study from others mentioned in the literature where comfort was a limiting factor. This high level of tolerability is likely due to the specific pulse configuration and the use of FES current.

5.7 STUDY LIMITATIONS

The primary limitation of this study was the difficulty in participant recruitment due to the extremely strict inclusion and exclusion criteria, designed to isolate vascular reactivity. Additionally, the COVID-19 pandemic imposed significant logistical challenges, impacting the pace of data collection and study execution.

6. CONCLUSION

This pilot study demonstrated that NMES significantly altered vessel diameters across all evaluated segments and increased blood flow velocities in the lower limbs. Specifically, significant increases in Peak Systolic Velocity (PSV) and Peak Velocity were observed in the femoral vessels, while numerical but non-significant increments were noted in the popliteal territory. These hemodynamic changes were observed in both the stimulated and non-stimulated limbs (with the exception of popliteal vein flow volume), suggesting a systemic effect of the intervention. Furthermore, the protocol induced a numerical, non-significant increase in tissue plasminogen activator (tPA) levels. The NMES parameters used in this study proved to be a safe and exceptionally comfortable circulatory stimulus for healthy subjects.

Declarations

Declaration of interest:

None

Author Contribution

Conception J.V.O.M.Design of the work J.V.O.M.Acquisition, analysis J.V.O.M., M.B., L.P.J., R.G.J., R.E.F.P, W.B.Y., M.L.S.interpretation of data J.V.O.M., L.P.J., M.L.S.have drafted the work or substantively revised it J.V.O.M., M.B., L.P.J., R.G.J., R.E.F.P, W.B.Y., F.H.A.M., M.L.S.Have approved the submitted version (and any substantially modified version that involves the author's contribution to the study) J.V.O.M., M.B., L.P.J., R.G.J., R.E.F.P, W.B.Y., F.H.A.M., M.L.S.AND to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. J.V.O.M., M.B., L.P.J., R.G.J., R.E.F.P, W.B.Y., F.H.A.M., M.L.S.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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