Received: 13 April 2023 | Accepted: 23 August 2023

DOI: 10.1111/eci.14091

ORIGINAL ARTICLE

WILEY

Blood flow effects of percutaneous peripheral nerve stimulation. A blinded, randomized clinical trial

Nuria Viudes-Sarrión[1,2,3] | **Fernando Aleixandre-Carrera**[3,4] | **Patricia Beltrá**[3,5] | **Francisco Javier Ortega**[6,7] | **Francisco Javier Molina-Payá**[7] | **Enrique Velasco**[3,8] | **Miguel Delicado-Miralles**[3,4]

[1]Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium

[2]Human Movement Biomechanics Research Group, Deptartment of Movement Sciences, KU Leuven, Leuven, Belgium

[3]Neuroscience in Physiotherapy (NiP), Independent Research Group, Elche, Spain

[4]Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, San Juan de Alicante, Spain

[5]Physical Therapy Department, Valencia University, Valencia, Spain

[6]Physical therapy and advanced rehabilitation clinic RehAv Elche, Elche, Spain

[7]Physical Therapy Department, Health Sciences Faculty, CEU-Cardenal Herrera University, CEU Universities, Elche, Spain

[8]Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven; VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium

Correspondence
Miguel Delicado-Miralles and Enrique Velasco, Neuroscience in Physiotherapy (NiP), Independent Research Group, Elche, Spain.
Email: mdelicado@umh.es and e.velascoserna@gmail.com

Abstract

Background: The vasculature function is mainly regulated by the autonomic nervous system. Importantly, the sensory-motor nervous system also innervates peripheral vessels and has the capacity to modulate vascular tone. Here we investigated the effects of electrical stimulation of a mixed nerve trunk on blood flow in deep arteries and muscle perfusion. Our hypothesis is that stimulation of a mixed nerve can modify blood flow.

Methods: Twenty-nine healthy participants were included into a randomized-crossover and blinded clinical trial. Each subject received a placebo and two percutaneous peripheral nerve stimulation (pPNS) protocols on the median nerve: Pain Threshold continuous Low Frequency (PT-cLF) and Sensory Threshold burst High Frequency (ST-bHF). Blood flow was then assessed bilaterally using Power Doppler Ultrasonography at the main arteries of the arm, and blood perfusion at the forearm muscles. Afterwards, blood flow was quantified using a semi-automatized software, freely shared here.

Results: Placebo, consisting in needle insertion, produced an immediate and generalized reduction on peak systolic velocity in all arteries. Although nerve stimulation produced mainly no effects, some significant differences were found: both protocols increased the relative perfusion area of the forearm muscles, the ST-bHF protocol prevented the reduction in peak systolic velocity and TAMEAN of the radial artery produced by the control protocol and PT-cLF produced a TAMEAN reduction of the ulnar artery.

Conclusions: Therefore, the arterial blood flow in the arm is mainly impervious to the electrical stimulation of the median nerve, composed by autonomic and sensory-motor axons, although it produces mild modifications in the forearm muscles perfusion.

Nuria Viudes-Sarrión and Fernando Aleixandre-Carrera contributed equally to this work and share first authorship.

KEYWORDS
arterial blood flow, electrical nerve stimulation, muscle perfusion, percutaneous peripheral nerve stimulation, power Doppler ultrasound, vascular physiology

1 | INTRODUCTION

Percutaneous peripheral nerve stimulation (pPNS) is a popular technique used for pain relief.[1] It consists of inserting a sterile blunt-tipped needle close to a peripheral nerve and delivering electrical current to modify its activity. Although pPNS has been shown to induce pain relief,[1] its effects over the blood flow are not explored.

Traditionally, the autonomic nervous system is considered the main regulator of blood flow, a role mediated by the release of neurotransmitters from the nerve, such as acetylcholine or norepinephrine, that diffuse to the blood vessel and regulate tone and contractility.[2] However, recent evidence demonstrates that sensory axons pertaining to peripheral sensory neurons also present the capability to modulate the vascular tone, through the antidromic release of substance P and calcitonin gene-related peptide (CGRP) from the perivascular afferent innervation.[3] In this regard, it has been reported that trigeminal pPNS can increase cerebral blood flow in an animal model of cerebral vasospasm.[4] Moreover, some studies found that transcutaneous electrical nerve stimulation (TENS) affects skin perfusion.[5–7]

Given the implication of both autonomic and sensory axons in vascular regulation, and the rising use of techniques like pPNS for pain relief, it is surprising that the vascular effects of stimulating a main nerve trunk remain unexplored. Studying the vascular effects of pPNS would enhance our knowledge about the relationship between the vascular and nervous systems. Additionally, this information has direct clinical implications, as it can identify potential vascular side effects, or benefits, of pPNS applications. This is especially important for patients with conditions like diabetes, peripheral vascular disease[8] and in patients at risk of thrombosis, where altered blood flow can increase the risk of thrombogenesis.[9] Therefore, the aim of this work was to investigate the effects of pPNS on blood flow in deep structures. To this end, we studied the effects on arterial blood flow and muscle perfusion of two pPNS protocols, for which both pain relief and motor effects have been previously reported.[10] pPNS and a placebo intervention were applied over the median nerve of healthy subjects, and blood flow was assessed using power Doppler ultrasonography. Our hypothesis is that pPNS will modify blood flow in deep structures, due to the close interaction between autonomic and sensory innervation and the vascular function of the blood vessels.

2 | MATERIALS AND METHODS

2.1 | Study design

A blinded, randomized-crossover design of repeated measures was used for this clinical trial. All volunteers received three protocols in a randomized order: a control intervention (no stimulation) and two pPNS protocols. Consecutive treatment sessions were spaced at least 1 week to avoid cross-effects. Interventions were applied in the upper limb, also randomized and blinded for each subject. The statistician randomized the assignment order with Excel (Block randomization).

2.2 | Participants

Twenty-nine young and healthy subjects (11 women, 23 years old [SD 2.24]) were initially recruited (Figure 1). The exclusion criteria were as follows: being physically inactive (<150 min of moderate-intensity activity per week); upper limb pathology; any disease discouraging electrical stimulation and/or needle insertion such as coagulation deficits or belonephobia; any pathological condition such as immunodepression, chronic pain, circulatory problems or neurological disease[11,12]; taking anticoagulants or pharmacological pain treatment (NSAIDs <24 h or opioids); being a professional athlete or being pregnant.[10,13]

Participants signed an informed consent in accordance with the Helsinki Declaration. This study was approved by the Ethical Committee of Pharmacological Research in the General University Hospital of Elche, Alicante, Spain, and preregistered in clinicaltrials.gov (NCT04475133). Data are publicly available at DOI: 10.17605/OSF.IO/NVRW8.

2.3 | Intervention

An electro-stimulator device (EPTE2 BIPOLAR SYSTEM by IONCLINICS SL., Valencia, Spain) was used to apply pPNS at the median nerve. A non-bevelled, blunt-tipped needle (0.16 × 25 mm, steel material, IONCLINICS SL.) was inserted on the mid-third of the arm, lateral to the median nerve and medial to the biceps muscle (Figure 2A). An ultrasound-guided approach was used by a trained physical therapist to minimize the risk of damaging other

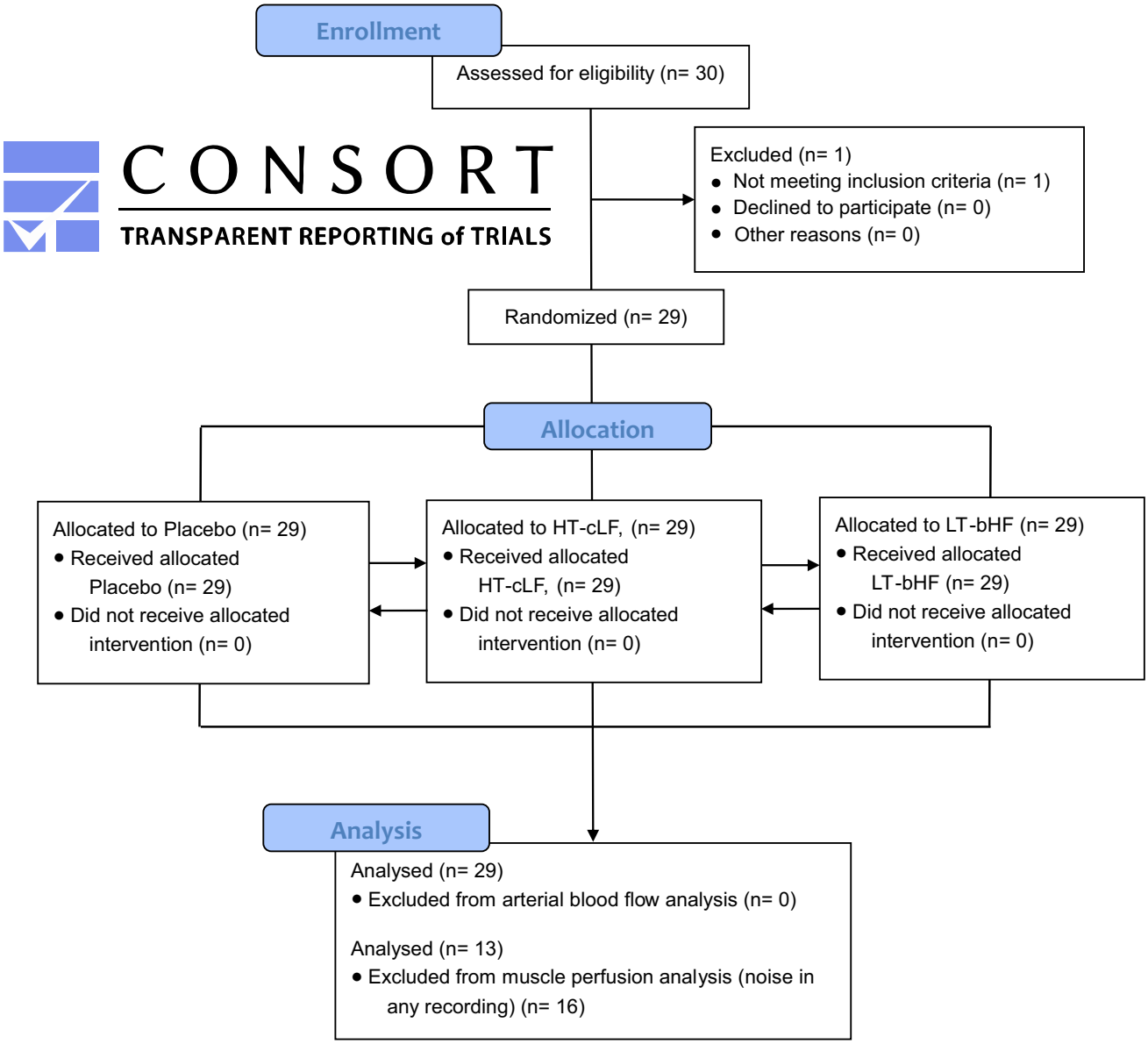
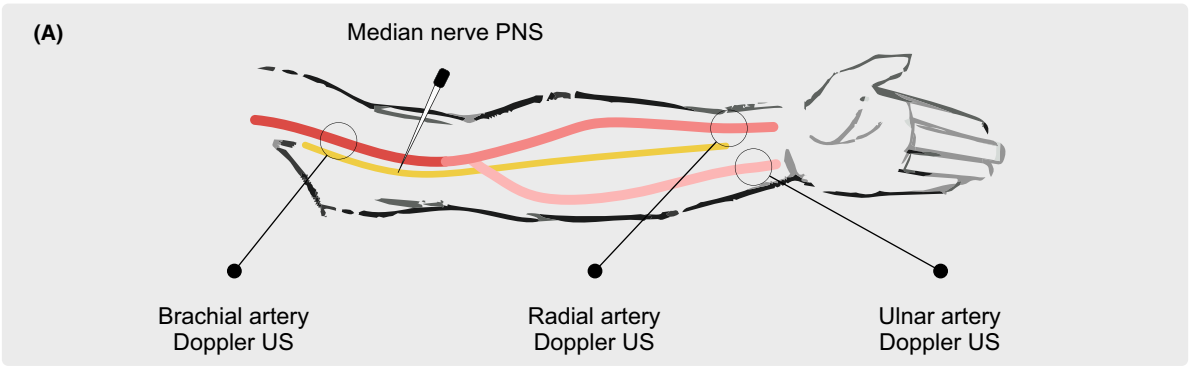


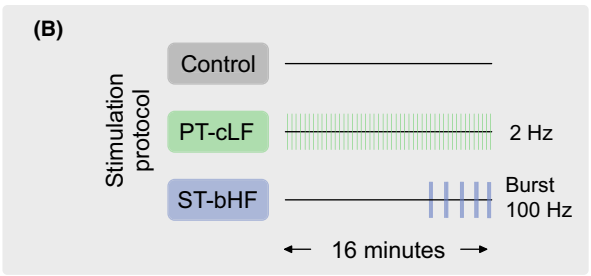
FIGURE 1 Consort flow diagram for dropouts and sample management. All subjects received all three protocols, and a single dropout was produced prior to assignation due to daily intake of NSAIDs. Thirteen subjects were excluded from muscle perfusion analysis due to noise presence.

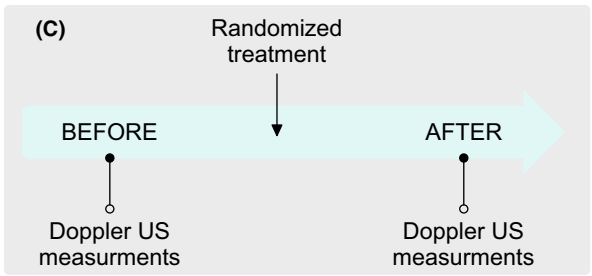
structures and optimize electrode positioning. A 5 × 5 cm surface adhesive electrode placed over the acromioclavicular joint completed the circuit. Subjects were in a comfortable lateral position during the procedure.

The control protocol consisted of needle insertion without current application, even though the electrostimulator was turned on and showed the exact same signs of functioning. *P*ain *T*hreshold *c*ontinuous *L*ow *F*requency (PT-cLF) stimulation consisted in the application of 250 ms pulses of a biphasic, symmetrical, squared current at 2 Hz with current intensity adjusted to pain threshold, to ensure an activation of nociceptive neurons. Importantly, this intensity was sufficient to produce muscle contraction in most cases. The *S*ensory *T*hreshold *b*urst *H*igh *F*requency (ST-bHF) stimulation consisted of five bursts of 100 Hz stimulation for 5 s separated by 55 s (summing a total treatment time of 5 min). The PT-cLF was the longest stimulation (16 min). Therefore, we adjusted the other protocols to a 16-min duration to maintain the subjects' blinding. Specifically, to adjust the ST-bHF protocol to 16 min, the current intensity was 0 during the first 11 min of intervention[10] (Figure 2B), and then the protocol, of 5 min duration, started. The absolute intensity thresholds necessary to cause the desired perceptions using these protocols have been previously reported in Beltrá et al.[10]

Following every intervention, the researcher applied pressure to the needle insertion site for 1 min to prevent







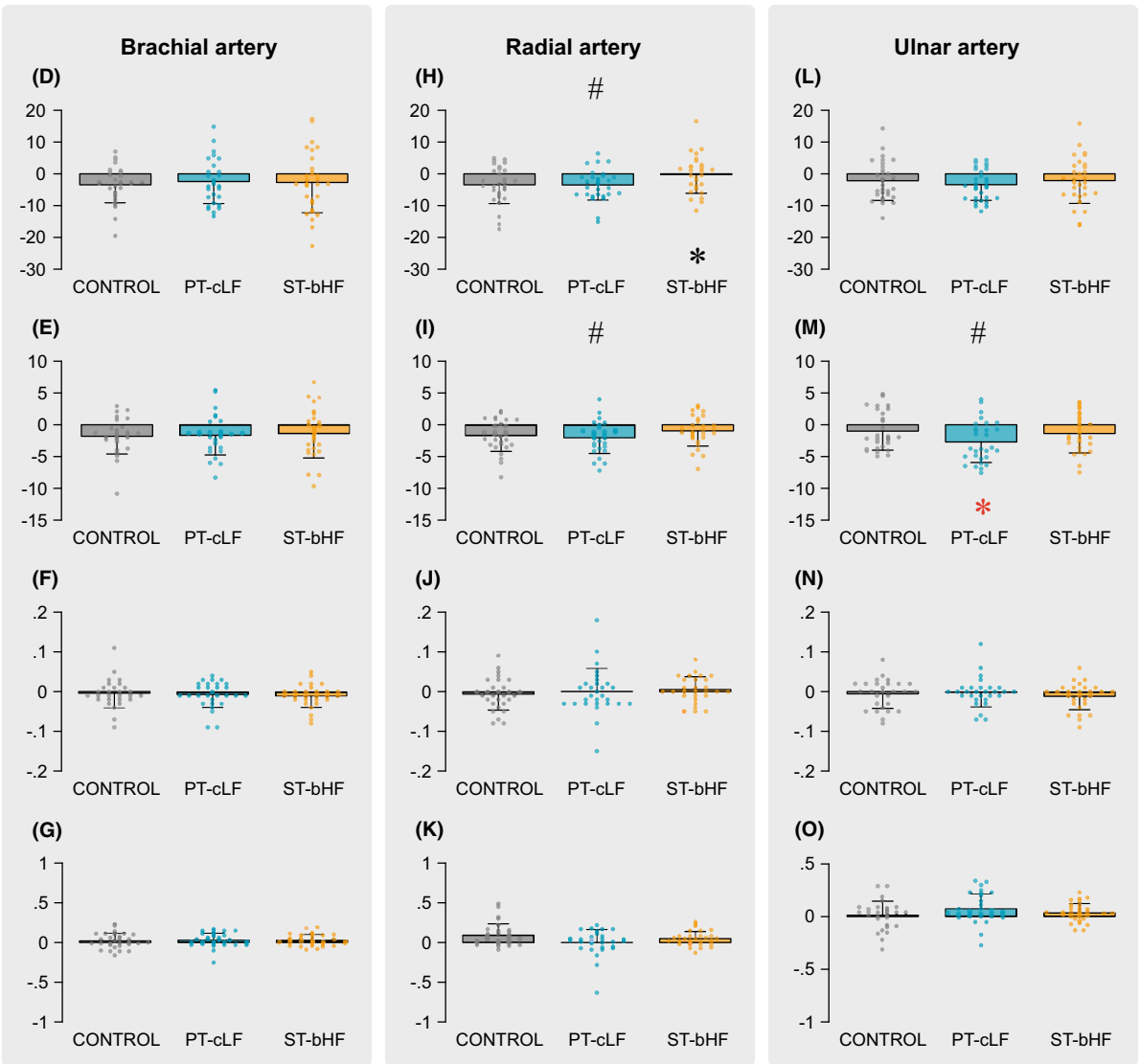


FIGURE 2 Summary of the experimental design and the effects of median nerve pPNS on ipsilateral arm blood flow. (A) Illustration depicting the placement of the active electrode and the Doppler measurements at brachial, radial, and ulnar arteries. (B) Representation of the protocols used: Control group received needle insertion, but no current. PT-cLF group received squared, biphasic, and symmetrical current at 2 Hz adjusted to pain threshold. ST-bHF received 5 burst of 100 Hz for 5 s spaced by 55 s, adjusted to sensory threshold. (C) Doppler US measurements were collected before and after each intervention. (D–O) Mean difference of peak systolic velocity (PSV), time-averaged medium velocity (TAMEAN), cross-sectional area (CSA) and resistance index (RI) for brachial (D–G), radial (H-K) and ulnar arteries (L–O). All bars represent posttreatment mean difference ± SD. For the secondary analysis, we used an ANCOVA linear model based on gaussian distribution with Identity link for brachial, based on gamma distribution with Identity link for radial and based on gaussian distribution with log link for ulnar artery. # in the upper part of the figure denotes statistical significance on Repeated Measures ANOVA and the * has been used to represent the *post hoc* comparisons (<0.05). The same symbols, but in red colour (# and *), have been used to represent the secondary analysis.

bleeding, examined the skin and asked participants about any adverse effects. Participants were also asked to identify the control intervention to assess the blinding protocol.

2.4 | Outcome measures

Participants completed a demographic questionnaire, which included gender, age, height, weight, physical activity, smoking or drinking habits and drug consumption. Experiments took place in a sound-attenuated and temperature-controlled laboratory.

Power Doppler ultrasonography was performed with a high-resolution ultrasonogram (LOGIQ S7 Expert/Pro, Soma Tech Intl, *Bloomfield*, EEUU) using a multifrequency linear transducer (7–14 MHz). The probe position was standardized with a mark on the skin and a constant angle of insonation <60°. Each measurement lasted ≥15 s and was repeated before and after each pPNS protocol. An additional measurement was taken after needle insertion in the control protocol to measure its immediate effect. The evaluator could not be blinded for this additional control measurement, but evaluator bias due to interpretation of Doppler images in real-time was unlikely due to the complex processing required.

2.4.1 | Upper limb arterial Doppler

Blood flow was assessed bilaterally at the brachial artery (2–3 cm above needle placement) and unilaterally at the ulnar and radial arteries (3 cm above wrist level). Power Doppler mode was used to localize the arteries with parameters fixed for each subject to ensure intrasubject comparability (frequency = 8.3–8.9 Hz; wall filter = 40–60 Hz; pulse repetition frequency = 11.9–20.8 Hz or 8.9–14.9 Hz, for brachial or ulnar and radial arteries respectively). The gain was adjusted for each participant. Spectral Doppler mode was used to measure arterial blood flow, with three to four recordings per location averaged and the mean taken from the two most representative recordings. The variables analysed were as follows: peak systole velocity (PSV), time-averaged medium velocity (TAMEAN), cross-sectional area (CSA) and resistance index (RI). PSV at brachial artery was the primary variable.

2.4.2 | Forearm muscle perfusion Doppler

Muscle perfusion was measured transversally for 20 s in both arms, in a randomized order, at the junction of the proximal and middle thirds of the anterior compartment of the forearm.[12] The area with the highest power Doppler signal (DS) was identified. Doppler settings were optimized for detection of intramuscular blood flow by adjusting frequency (750–770 Hz), pulse repetition frequency (1000 Hz), gain (just below the level that produced background noise, typically 20–25), and a medium wall filter (120–150 Hz). The colour box was adjusted to include the largest muscle area. To standardize the pressure applied over the probe, the researcher assured that there was a discernible thin layer of gel between the transducer and the skin.

Commercial ultrasound systems do not quantify muscle perfusion,[12] resulting in qualitative descriptions in the literature.[14,15] It is less common to find studies in which the muscle perfusion analysis is quantitative,[12,16] as it is time-consuming. To semi-automate DS quantification and data extraction, we developed two customized software programs based on previous works. First, we supervised and discarded videos with low signal-to-noise ratio, where noise was identified as incoherent spatial or temporal DS.[17]

The variables extracted were as follows: (1) area of the colour box (cm^2); (2) number of DS; (3) relative perfusion area (RPA), percentage of pixels with DS in the colour box; and (4) estimated fractional moving blood volume

(EFMBV), the amount of detectable moving blood.[16] For details on the software and how to use it, see Supporting Information (DOI: 10.17605/OSF.IO/NVRW8.).

2.5 | Sample size calculation

Sample size was calculated based on a previous work,[18] resulting in 10 subjects with an alpha error of 5%, 80% statistical power and an effect size of $f=2.1$ for the primary outcome (PSV at brachial artery) using GPower.[19]

However, since this work was part of a larger clinical trial project measuring other variables, we recalculated the sample size for the primary outcome ($f=0.35$, mechanical punctate pain threshold of the third fingertip[10]). We obtained a sample size of 25 subjects. In prevision of possible dropouts, we added 20% of subjects to the sample (+5), reaching a sample of 30 subjects. This sample size is sufficient for the primary outcome and the variables reported in this study.

2.6 | Statistical analysis

Statistical analyses were preregistered on clinicaltrials.gov and performed using IBM SPSS Statistics (Version 26.0; IBM Corp.). Normality was tested using Shapiro–Wilk test and density histograms. Data are represented as mean difference and SD in all figures.

To examine the effect of the control protocol, we compared pre- versus post-intervention measurements (paired *t*-test or Wilcoxon's test). To compare between protocols, data were normalized to each day baseline through subtraction (Figure 2C). We analysed the effect of the three interventions using repeated measures ANOVA (rmANOVA) or non-parametric Friedman's test, depending on normality assumption. Paired *post hoc* comparisons were made using *t*-tests or Wilcoxon's test, adjusted for multiple comparisons by Bonferroni method. An *ad hoc* ANCOVA model generalized to a Gaussian, gamma or inverse Gaussian distribution, depending on data distribution. This analysis, not preregistered, was performed for additional exploratory analysis with higher statistical power.[20]

3 | RESULTS

Recruitment ended in November 2020 (started in August). One participant dropped out before allocation due to NSAID consumption prior to the experiment. The descriptive data of 29 participants are summarized in Table 1. For the perfusion analysis, the sample size was reduced to 13 subjects due to noise in some recordings but remained above the required sample size of 10 subjects (Figure 1). Tables 2 and 3 present the raw data of differences, *p*-values and effect size from rmANOVAs to aid text reading. Raw data of individual values are reported in Tables S1 andS2, and all data are available in the OSF repository (Supporting Information, DOI 10.17605/OSF.IO/NVRW8.). The conclusions drawn from ad hoc ANCOVAs analysis support and complement the interpretation of the data obtained from the main analysis.

3.1 | pPNS effects on the upper limb arterial Doppler

3.1.1 | Brachial artery

First we assessed whether the control intervention, a needle insertion without electrical stimulation near the median nerve at brachial level, could affect brachial artery blood flow. We observed a reduction in peak systolic velocity (PSV) ($-3.4\,cm\,s^{-1}$, SD 5.7, $p=.003$, paired *t*-test, Cohen's $d=.38$), time-averaged medium velocity (TAMEAN) ($-1.8\,cm\,s^{-1}$, SD 2.8, $p=.002$, $d=.45$) and an increase in RI (0.8 au, SD 2.2, $p=.054$, $d=.39$) after control protocol. The same effects were observed in the electrical stimulation protocols, with no significant differences between them or against the control intervention (Table 2). These results were confirmed by our *ad hoc* analysis (Table 2) (Figure 2D–G).

TABLE 1 Descriptive variables of the sample.

Descriptive data ($n=29$)			
Quantitative (Mean (SD))		**Qualitative (frequency)**	
Age	23 (2.4)	Gender (women/men)	11/18
Height (cm)	170.7 (9)	Smoker	8
Weight (Kg)	67.5 (11.7)	Occasionally or weekly drinker	29
Body mass index	23.1 (3.1)	Previous invasive treatment	24
		Exercise frequency (>3/week)	27

Note: Bold values are statistical significance.

TABLE 2 pPNS protocols effects over arterial blood flow variables.

		Intervention (Δmean (SD))			Repeated measures ANOVA			Generalized model	
Artery	**Variable**	**Control**	**PT-cLF**	**ST-bHF**	**F**	***p***	**ηp2**	**R^2**	***p***
Brachial	PSV	−3.4 (5.7)	−2.4 (6.9)	−2.7 (9.4)	.16	.853	.005	.52	.812
	TAMEAN	−2 (2.9)	−1.8 (3.2)	−1.5 (3.8)	.21	.814	.008	.23	.803
	CSA	−.61 (3.2)	−.01 (.03)	−.01 (.03)	.54	.592	.043	.03	.400
	RI	.02 (.09)	.028 (.09)	.027 (.07)	.09	.906	.003	.14	.979
Radial	PSV	−3.4 (5.7)	−3.4 (4.8)	−.23 (5.9)	**4.51**	**.015***	**.135**	**.60**	**.042****
	TAMEAN	−1.9 (2.5)	−2 (2.69)	−1.2 (2.3)	1.08	.347	.047	**.7**	**.041****
	CSA	−.009 (.04)	.0005 (.06)	.0069 (.03)	.75	.480	.029	.04	.648
	RI	092 (.14)	.003 (.16)	.05 (.09)	2.33	.116	.143	.04	.417
Ulnar	PSV	−2.1 (6.1)	−3.4 (4.9)	−2.1 (7.2)	.39	.676	.013	.5	.726
	TAMEAN	−.9 (3)	−3 (3.1)	−1.4 (3)	3.09	.054	.110	**.4**	**.048****
	CSA	−.008 (.04)	.0004 (.04)	.011 (.03)	.86	.431	.033	.03	.448
	RI	.14 (.13)	.074 (.14)	.36 (.09)	1.94	.153	.063	.14	.979
Contra-lateral Brachial	PSV	−3.1 (7.9)	−1.8 (7.2)	−2.6 (7.7)	.21	.808	.008	.48	.957
	TAMEAN	−1.3 (3.4)	−1.2 (1.9)	−2.7 (2.5)	2.99	.059	.107	**.4**	**.024****
	CSA	.001 (.03)	.001 (.03)	−.01 (.03)	2.17	.124	.080	.06	.431
	RI	.04 (.11)	.01 (.17)	.05 (.06)	.85	.434	.029	.08	.536

Note: Bold values are statistical significance.

Abbreviations: CSA, cross-sectional area; PSV, peak systole velocity; PT-cLF, pain threshold continuous low frequency; RI, resistance index; ST-bHF, sensory threshold burst high frequency; TAMEAN, time-averaged medium velocity.

*Denotes statistical differences in rmANOVA (<0.05).

**Denotes statistical differences in ANCOVA generalized model (<0.01).

3.1.2 | Radial and ulnar arteries

We evaluated the effect of pPNS on blood flow in the radial and ulnar arteries at wrist level. In the radial artery, needle insertion without electric stimulation diminished PSV and TAMEAN (−3.2 and −1.7 cm s^{-1}, SD 4.7 and 2.5, $p = .001$, $d = .43$ and .72, respectively) and increased RI (0.09 au, SD .14, $p = .001$, $d = 1.06$), similarly to the effects observed for the brachial artery. However, the ST-bHF protocol prevented PSV reduction observed in control protocol (3.2 cm s^{-1}, SD 7, $p = .018$, $d = .55$, ST-bHF vs. control). Additionally, the generalized ANCOVA analysis confirmed the ST-bHF effect over the PSV (Difference = 2.773 cm*s^{-1}, z-score = 2.2, $p = .025$). Furthermore, this analysis detected statistical differences due to intervention in the TAMEAN between ST-bHF and PT-cLF protocols (Difference = .039 cm*s^{-1}, z-score = 2.5, $p = .012$), but not respect to placebo.

Our main analysis detected no significant changes in the ulnar artery (ANOVA, Table 2). However, the increased power of our secondary analysis allowed to detect a significant difference, showing that PT-cLF decreased TAMEAN by 23% compared to control (95% CI = [.03, .43] %, z-score = 2.3, $p = .024$). In summary, ST-bHF prevented PSV and TAMEAN reduction in the radial artery, while PT-cLF reduced TAMEAN in the ulnar artery, and no other significant changes were observed (Figure 2H–O).

3.1.3 | Contralateral brachial artery

Interestingly, control intervention decreased PSV and TAMEAN compared to basal conditions also in the brachial artery of the non-intervened arm (3 and 1.4 cm s^{-1}, SD 7.7 and 3.2, $p = .043$ and .031, $d = .35$ and .48, respectively). This suggests that the effect of needle insertion is produced in a generalized way, not only in the territory innervated by the stimulated nerve. Additionally, we have analysed the time course of the blood flow changes observed after control protocol and found that they were produced immediately after needle insertion (Supporting Information, DOI: 10.17605/OSF.IO/NVRW8.).

Regarding the electrical stimulation protocols, the main ANOVA analysis found that none of the stimulation protocols effects differed from the ones produced by the control intervention (Figure 3). Contrary, our secondary analysis, due to greater statistical power, found that ST-bHF reduced TAMEAN compared to control protocol (Difference = −1.8 cm*s^{-1}, z-score = 2.9, $p = .003$).

TABLE 3 pPNS protocols effects over forearm muscle perfusion.

			Intervention (Δmean (SD))			Repeated measures ANOVA			Generalized model	
Arm	**Variable**		**Control**	**PT-cLF**	**ST-bHF**	**F**	***p***	**ηp2**	**R^2**	***p***
Ipsi	Box size		−.17 (.11)	−.11 (.2)	−.16 (.14)	1.07	.360	.08	.42	.113
	Systole	EFMBV	.79 (1.6)	.75 (1.2)	.36 (2.6)	.19	.825	.01	.17	.654
		RPA	.23 (2.4)	.95 (2.6)	1.3 (1.9)	.68	.516	.05	**.59**	**.001****
		N° of DS	−.5 (2)	−1.2 (2)	1.1 (2.7)	**4.38**	**.024***	**.26**	.08	.462
	Diastole	EFMBV	2 (5.9)	3.8 (7.4)	−2.9 (14)	1.59	.225	.11	.09	.200
		RPA	−.08 (1.5)	−.47 (1)	.34 (1.4)	.98	.389	.07	.09	.322
		N° of DS	−.19 (1.5)	−.69 (1.7)	.55 (2.5)	1.10	.348	.08	.2	.766
Contra	Box size		−.19 (.1)	−.07 (.023)	−.16 (.15)	1.67	.233	.23	**.39**	**.006****
	Systole	EFMBV	.0001 (3)	.1 (1.4)	−.5 (1.5)	.31	.739	.02	.27	.328
		RPA	.79 (2.2)	.64 (2)	.84 (1.4)	.06	.941	.005	.29	.736
		N° of DS	.4 (2.8)	.34 (3.4)	.34 (2.5)	.002	.967	.001	.07	.578
	Diastole	EFMBV	−1.7 (12.3)	6.1 (12.5)	7.6 (10.1)	2.50	.103	.17	.04	.333
		RPA	.1 (1.9)	.27 (1.1)	.65 (1.2)	.64	.532	.05	.16	.633
		N° of DS	−.17 (2.1)	.96 (2.7)	.62 (2.2)	1.04	.368	.08	.26	.580

Note: Bold values are statistical significance.

Abbreviations: EFMBV, estimated fractional moving blood volume; N° of DS, number of Doppler signals; PT-cLF, pain threshold continuous low frequency; RPA, relative perfusion area; ST-bHF, sensory threshold burst high frequency.

*Denotes statistical differences in rmANOVA (<0.05); **Denotes statistical differences in ANCOVA generalized model (<0.01).

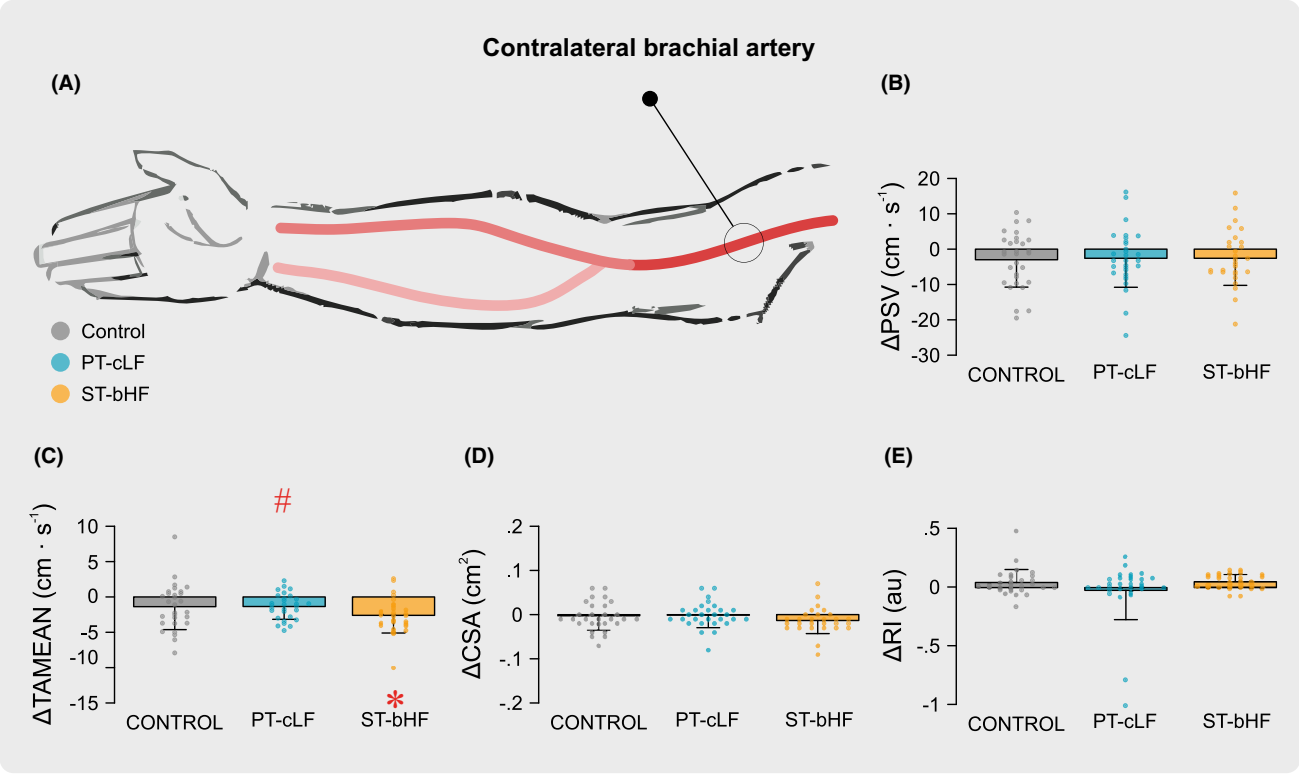


FIGURE 3 Effects of median nerve pPNS on contralateral arm blood flow. (A) Doppler US was measured at the contralateral brachial artery exclusively. (B) Mean difference of peak systolic velocity (PSV). (C) Mean difference of time-averaged medium velocity (TAMEAN). (D) Mean difference of cross-sectional area (CSA). (E) Mean difference of resistance index (RI). All bars represent posttreatment mean difference ± SD. #, in red, in the upper part of the figure denotes statistical significance on the secondary analysis (ANCOVA generalized model) and the *, in red, has been used to represent the *post hoc* comparisons.

3.2 | pPNS effects on forearm muscle perfusion

We used a custom software to obtain the muscle perfusion signal (Figure 4A,B). Regarding the ipsilateral arm, the control protocol did not alter any of the muscle perfusion variables evaluated. Otherwise, our main ANOVA analysis detected significant differences in the number of individual DS between protocols (Table 3). However, the *post hoc* comparisons were unable to clarify between which groups these differences are being produced ($p > .05$) (Figure 4C–H), although looking at the averages of the groups, the greater differences are observed between both stimulation protocols (Table 3). Accordingly, our secondary analysis did not find significant differences in DS (Table 3). However, the secondary analysis revealed that both ST-bHF and PT-cLF increased the relative perfusion area (RPA) on the forearm muscle perfusion, compared to control (Difference = .097 and .072%, z-score = 2.7 and 2, $p = .009$ and .048, respectively).

When the contralateral arm was assessed, the control protocol neither changed EFMBV, RPA or the number of DS detected during the systole (.2%, −.3% and .3%, SD 2.3, 2 and 2.5, $p = .667$, .420 and .531) and diastole (1.9%, −.1% and −.03%, SD 14.6, 1.6 and 1.7, $p = .531$, .805 and .923). In this case, no statistical difference were found by the main analysis (Table 3). However, our secondary analysis found that the box size on contralateral arm was different between control and PT-cLF protocols (Difference = .15 cm^2, z-score = 3.19, $p = .007$). This result could influence the signal recorded, although this difference is small and the results in other variables are negative (Figure 4I–N). In summary, the stimulation protocols produced mild alterations in muscle perfusion of the ipsilateral arm: the number of DS during the systolic peak in the ipsilateral arm was significantly different between protocols and both protocols induced an RPA increase. The rest of the variables were not altered by nerve stimulation and no contralateral effects were observed.

3.3 | Effectiveness of masking and adverse effects

After the intervention, subjects were asked to guess if they received the control or actual intervention. Out of 29, only 2 (7%) correctly identified the control intervention. This was surprising as electrical stimulation is generally difficult to mask. One week passed between interventions, which may have helped subjects forget the previous treatment. We (authors) also tested the protocols on ourselves and found that current stimulation was clearly perceived during intervention, but also some tingling sensation was perceived during the control protocol, maybe due to the close location between the needle and the nerve. Mild adverse effects were reported by some subjects, including nuisance from needle insertion (3 out of 87 interventions) and current application (2 out of 87 interventions), as well as minor hematoma (3 out of 87 interventions). The probability of suffering a minor adverse effect after pPNS intervention was .09%. No severe adverse effects were reported.

4 | DISCUSSION

In this study, we investigated, for the first time, the effect of pPNS on arterial blood flow and muscle perfusion in the upper limb of young healthy subjects. The results of the present study are mainly negative. However, some effects were produced by our protocols: ST-bHF affected blood flow of the radial artery, a territory innervated by median nerve, meanwhile PT-cLF did affect the blood flow of ulnar artery. Moreover, both stimulation protocols did increase the relative perfusion area (RPA) of the forearm muscle, innervated by the median nerve. We are also providing a datasheet containing our data and software scripts used for muscle perfusion analysis, along with instructions for use.

As the median nerve is a mixed nerve, the mildness of the vascular effects produced by its stimulation is surprising. Physiologically, there are several plausible explanations. PT-cLF is a conventional transcutaneous and percutaneous stimulation protocol for pain relief, while ST-bHF is a new approach based on stimulation protocols for central nervous system synaptic plasticity.[10] One possibility is that the low intensity of ST-bHF protocol failed to activate high threshold C-fibres, which contribute to vasodilation through antidromic release of CGRP[3,21] and catecholamines release.[22] Another plausible explanation is that TENS currents below motor threshold are unable to increase blood flow.[5,11,23] In healthy individuals, percutaneous stimulation of the common peroneal nerve can increase venous flow to the leg through the contraction of leg muscles.[13]

Although the PT-cLF protocol was able to activate both low and high threshold sensory neurons, its frequency was lower compared to the vasodilation inducing protocol (10 Hz) used in another study.[3] It is possible that the PT-cLF protocol may have depressed the sensory pathway,[24] including autonomic reflex responses, preventing blood flow changes. However, simply attributing the lack of effect of the ST-bHF protocol to its low intensity or the PT-cLF protocol to its low frequency seems a circular argument to us. To explore these possibilities, a high frequency and high intensity protocol should be tested. It is

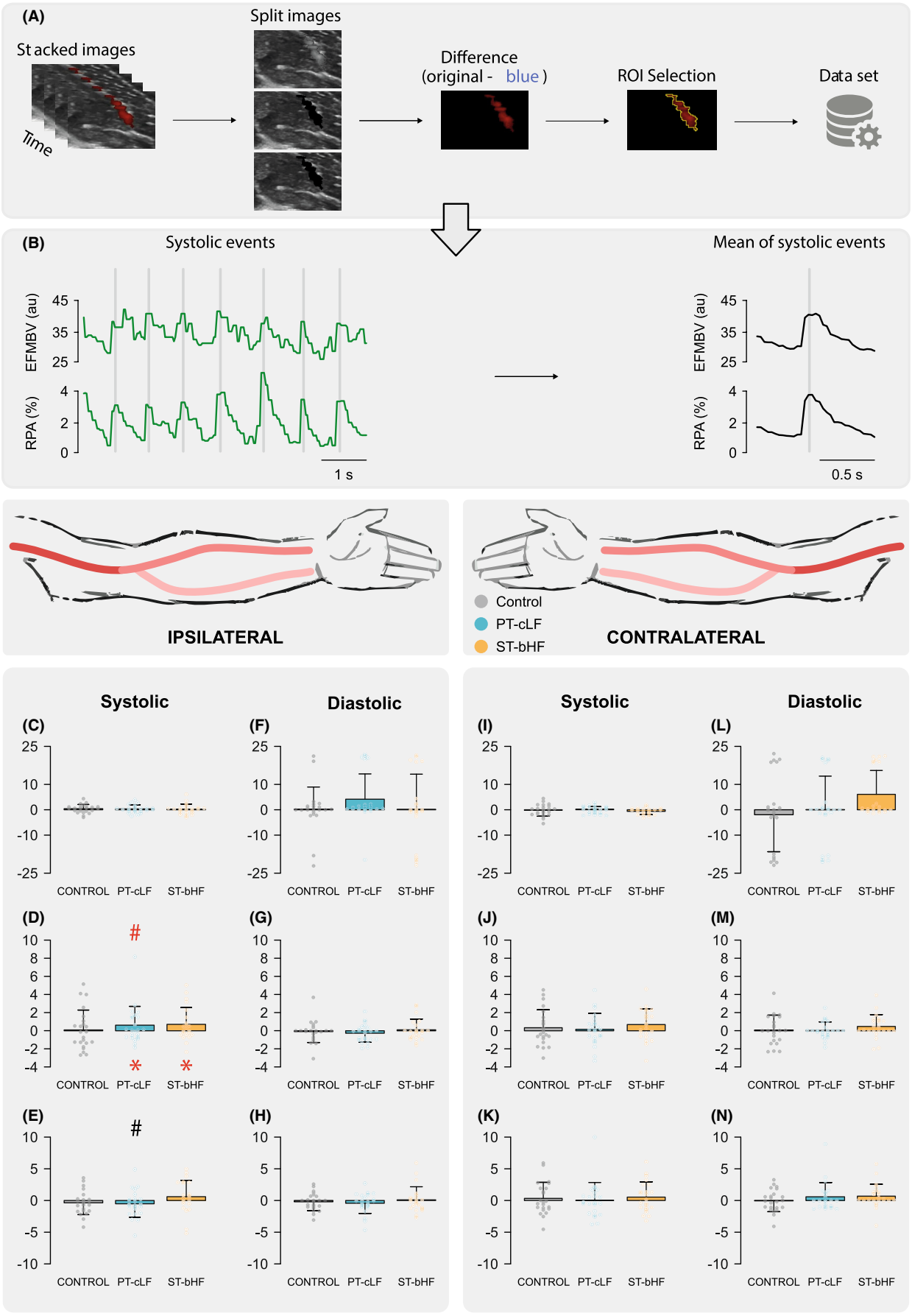


FIGURE 4 Effects of median nerve pPNS on forearm muscle perfusion. (A) Doppler US images were processed to obtain changes in intramuscular perfusion of the ipsilateral and contralateral forearm. (B) Systolic and diastolic events were detected and averaged using *Spike 2 v8.02*. (C–H) Mean difference of the estimated fractional moving blood volume (EFMBV), relative perfusion area (RPA) and the number of Doppler signals (#DS) in ipsilateral forearm during systole and diastole, respectively. (I–N) Mean difference of EFMBV RPA and #DS in contralateral forearm during systole and diastole, respectively. All bars represent posttreatment mean difference ± SD. For the secondary analysis we used an ANCOVA linear model based on gaussian distribution with Identity link. #, in red, in the upper part of the figure denotes statistical significance on the secondary analysis (ANCOVA generalized model) and the *, in red, has been used to represent the *post hoc* comparisons.

worth noting that other studies have reported no effect of TENS or electro-acupuncture.[5,23]

Another possibility for the PT-cLF protocol is that it may be activating blood vessel-related fibres, but due to the unspecific nature of electrical stimulation and the high intensity of the protocol, both cholinergic and catecholaminergic neurons may be activated, leading to opposing functions that cancel each other out and produce no detectable effects.[22]

This study also found a robust reduction in arterial blood flow in all arteries immediately after needle insertion, which was maintained during the control intervention for 16 min. This contrasts with reports from other studies that have found an increase in cutaneous blood flow after needle insertion.[25,26] They proposed that needle insertion produces an immediate sympathetic nervous system activation, increasing blood flow. However, this theoretical sympathetic activity increase may also increase vascular resistance, limiting bloodstream through the blood vessels[27] as we report here. This generalized sympathetic activation also explains the globality of the blood flow reduction, that affects even the non-stimulated arm. Another possible explanation is the activation of a new population of peripheral perivascular neurons that cause vasoconstriction upon mechanical stimulation.[28] In this line, the ST-bHF protocol prevented peak systolic velocity and TAMEAN reduction in the radial artery, indicating that the effects of peripheral perivascular nerve stimulation on blood flow may depend on the innervation territory.[3] Accordingly, the proximal third of radial artery is partially innervated by the median nerve.[29] Previous studies have also reported increased muscle blood flow when specific dorsal root ganglia are stimulated.[3]

ST-bHF protocol reduced TAMEAN on contralateral brachial artery. Contralateral effects of ST-bHF suggest that some of the stimulation effects are not territory-specific but are generalized beyond the area innervated by the nerve. This effect is biologically plausible, and in line with a previous paper that found temperature changes in the contralateral limb to the one stimulated.[30] However, it should be considered that this TAMEAN reduction is not accompanied by changes in other blood flow variables in the contralateral limb and was only detected by our secondary analysis.

Otherwise, PT-cLF reduced the TAMEAN of the ulnar artery. Among all variables and locations assessed, this was the only effect produced by the PT-cLF protocol. Additionally, this effect was only detected by our secondary analysis. Thus, our interpretation is that PT-cLF produced very mild changes in blood flow at deep tissues.

Our muscle perfusion analysis reveals effects on two variables from the ipsilateral forearm. First, a difference in the number of DS was detected by our main ANOVA analysis. However, the ANOVA post hoc comparisons and our secondary analysis with increased statistical power find no differences. Thus, the effect over the number of DS on forearm muscle perfusion seem spurious, or extremely anecdotical in the best case. Second, our secondary analysis detected that both protocols produced an RPA increase. However, this was not accompanied by an effect in EFMBV or the number of DS detected. Therefore, our interpretation is that although the total amount of blood remains the same (EFMBV), as well as the number of individual sources of flowing blood (DS), more area is displaying blood flow because of vasodilation of the intramuscular capillary produced by the electrical stimulation. Still, it should be considered that this effect was only detected by our secondary analysis and the effect size is modest.

We acknowledge the limitations of our work. The sample size for muscle perfusion was reduced due to noise in the video recordings, although it remained within the ranges of the required sample size. To prevent noise in future studies, a probe holder should be used to avoid movement-derived noise. Also, the lack of a control treatment without needle insertion prevents us from attributing the observed blood flow reduction exclusively to needle insertion, as a nocebo effect could have occurred. Furthermore, the PT-cLF protocol typically induces muscle contraction, which can increase blood flow.[5,6,23] However, we observed no such relevant changes in blood flow produced by PT-cLF, despite muscle contractions. Finally, we have not assessed the blood flow responses along time after the intervention, thus characterizing the acute effects produced by nerve stimulation. Replicating this study in patients with peripheral vascular disease or painful conditions would be interesting for future research. Also, it

could be interesting to measure deep and superficial perfusion and compare the pPNS effects over muscle and skin perfusion.

In conclusion, upper limb arterial blood flow and forearm muscle perfusion are generally unaffected by percutaneous electrical stimulation of the median nerve. Although the effects of the stimulation protocols were mainly negative, some differences were found. Both protocols increased the relative perfusion area of the forearm muscles. Further, the ST-bHF protocol prevented the reduction in peak systolic velocity and TAMEAN of the radial artery produced in the control protocol. Meanwhile, PT-cLF produced a TAMEAN reduction of the ulnar artery. Physiologically, this evidence suggests that the electrical stimulation of a mixed peripheral nerve trunk could modify muscle perfusion and blood flow at arteries innervated by the stimulated nerve, with some dependence on the stimulation parameters. However, those effects are mild and very isolated, specially, when considering what would be expected when stimulating a supposedly crucial regulator of the vascular tone, such as a main mixed nerve. Clinically, our data indicates that pPNS is a safe intervention in terms of secondary effects over the peripheral vascular system.

AUTHOR CONTRIBUTIONS

Miguel Delicado-Miralles and Enrique Velasco envisioned, designed, and coordinated the study. Nuria Viudes-Sarrión collected all data, contributed to study design and wrote the methods of the first draft of the manuscript. Francisco Javier Ortega and PB gave clinical advice for the design and implementation of the study, as well as executed the treatments. Francisco Javier Molina-Payá gave expert advice regarding Doppler signal acquisition, analysis, and interpretation. Fernando Aleixandre-Carrera and Miguel Delicado-Miralles interpreted the results, reviewed the bibliography, and wrote together the first draft of the manuscript, then supervised by Enrique Velasco. Fernando Aleixandre-Carrera also designed and generated the figures. All authors supervised and gave feedback for the last version of the manuscript.

ACKNOWLEDGEMENTS

We deeply appreciate the volunteers that participated in this study: this kind of gestures favour the advance of science. We also want to acknowledge the material contribution of IONCLINICS SL., who kindly lend us an EPTE 2.0 electrical stimulator to carry out this study.

FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

FJO. is a teacher and promoter of pPNS among physical therapists in Spain.

ORCID

Enrique Velasco https://orcid.org/0000-0001-7299-0750
Miguel Delicado-Miralles https://orcid.org/0000-0002-5181-6742

REFERENCES

1. Cohen S, Gilmore C, Kapural L, et al. Percutaneous peripheral nerve stimulation for pain reduction and improvements in functional outcomes in chronic low Back pain. *Mil med*. 2019;184(Suppl 1):537-541. doi:10.1093/MILMED/USY310
2. Aalkjaer C, Nilsson H, de Mey JGR. Sympathetic and sensorymotor nerves in peripheral small arteries. *Physiol Rev*. 2021;101(2):495-544. doi:10.1152/PHYSREV.00007.2020
3. Sato A, Sato Y, Shimura M, Uchida S. Calcitonin gene-related peptide produces skeletal muscle vasodilation following antidromic stimulation of unmyelinated afferents in the dorsal root in rats. *Neurosci Lett*. 2000;283(2):137-140. doi:10.1016/S0304-3940(00)00932-0
4. Li C, White TG, Shah KA, et al. Percutaneous trigeminal nerve stimulation induces cerebral vasodilation in a dose-dependent manner. *Neurosurgery*. 2021;88(6):E529-E536. doi:10.1093/NEUROS/NYAB053
5. Cramp FL, McCullough GR, Lowe AS, Walsh DM. Transcutaneous electric nerve stimulation: the effect of intensity on local and distal cutaneous blood flow and skin temperature in healthy subjects. *Arch Phys med Rehabil*. 2002;83(1):5-9. doi:10.1053/APMR.2002.27478
6. Sandberg ML, Sandberg MK, Dahl J. Blood flow changes in the trapezius muscle and overlying skin following transcutaneous electrical nerve stimulation. *Phys Ther*. 2007;87(8):1047-1055. doi:10.2522/PTJ.20060178
7. Kamali F, Mirkhani H, Nematollahi A, Heidari S, Moosavi E, Mohamadi M. The effect of transcutaneous electrical nerve stimulation of sympathetic ganglions and acupuncture points on distal blood flow. *J Acupunct Meridian Stud*. 2017;10(2):120-124. doi:10.1016/J.JAMS.2017.01.003
8. Thiruvoipati T, Kielhorn CE, Armstrong EJ. Peripheral artery disease in patients with diabetes: epidemiology, mechanisms, and outcomes. *World J Diabetes*. 2015;6(7):961-969. doi:10.4239/WJD.V6.I7.961
9. Stone J, Hangge P, Albadawi H, et al. Deep vein thrombosis: pathogenesis, diagnosis, and medical management. *Cardiovasc Diagn Ther*. 2017;7(3):S276-S284. doi:10.21037/CDT.2017.09.01
10. Beltrá P, Ruiz-del-Portal I, Ortega FJ, Valdesuso R, Delicado-Miralles M, Velasco E. Sensorimotor effects of plasticity-inducing percutaneous peripheral nerve stimulation protocols: a blinded, randomized clinical trial. *Eur J Pain*. 2022;26(5):1039-1055. doi:10.1002/EJP.1928
11. Cramp AFL, Gilsenan C, Lowe AS, Walsh DM. The effect of high- and low-frequency transcutaneous electrical nerve stimulation upon cutaneous blood flow and skin temperature in healthy subjects. *Clin Physiol*. 2000;20(2):150-157. doi:10.1046/J.1365-2281.2000.00240.X

12. Dori A, Abbasi H, Zaidman CM. Intramuscular blood flow quantification with power doppler ultrasonography. *Muscle Nerve*. 2016;54(5):872-878. doi:10.1002/MUS.25108
13. Tucker AT, Maass A, Bain DS, et al. Augmentation of venous, arterial and microvascular blood supply in the leg by isometric neuromuscular stimulation via the peroneal nerve. *Int J Angiol*. 2010;19(1):e31-e37. doi:10.1055/S-0031-1278361
14. Klauser A, Frauscher F, Schirmer M, et al. The value of contrast-enhanced color Doppler ultrasound in the detection of vascularization of finger joints in patients with rheumatoid arthritis. *Arthritis Rheum*. 2002;46(3):647-653. doi:10.1002/ART.10136
15. Shio K, Homma F, Kanno Y, et al. Doppler sonographic comparative study on usefulness of synovial vascularity between knee and metacarpophalangeal joints for evaluation of articular inflammation in patients with rheumatoid arthritis treated by infliximab. *Mod Rheumatol*. 2006;16(4):220-225. doi:10.1007/S10165-006-0488-0
16. Newman JS, Adler R, Rubin JM. Power Doppler sonography: use in measuring alterations in muscle blood volume after exercise. *AJR Am J Roentgenol*. 1997;168(6):1525-1530. doi:10.2214/AJR.168.6.9168718
17. Li YL, Hyun D, Abou-Elkacem L, Willmann JK, Dahl JJ. Visualization of small-diameter vessels by reduction of incoherent reverberation with coherent flow power Doppler. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2016;63(11):1878-1889. doi:10.1109/TUFFC.2016.2616112
18. Jin HK, Hwang TY, Cho SH. Effect of electrical stimulation on blood flow velocity and vessel size. *Open Medicine*. 2017;12(1):5-11. doi:10.1515/MED-2017-0002
19. Faul F, Erdfelder E, Lang AG, Buchner A. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*. Vol 39. Psychonomic Society Inc; 2007:175-191. doi:10.3758/BF03193146
20. Fife D. The eight steps of data analysis: a graphical framework to promote sound statistical analysis. *Perspect Psychol Sci*. 2020;15(4):1054-1075. doi:10.1177/1745691620917333
21. Sato-Suzuki I, Kagitani F, Uchida S. Somatosensory regulation of resting muscle blood flow and physical therapy. *Auton Neurosci*. 2019;220:102557. doi:10.1016/J.AUTNEU.2019.102557
22. Glatte P, Buchmann SJ, Hijazi MM, Illigens BMW, Siepmann T. Architecture of the cutaneous autonomic nervous system. *Front Neurol*. 2019;10:970. doi:10.3389/FNEUR.2019.00970/BIBTEX
23. Chen CC, Johnson MI, Mcdonough S, Cramp F. The effect of transcutaneous electrical nerve stimulation on local and distal cutaneous blood flow following a prolonged heat stimulus in healthy subjects. *Clin Physiol Funct Imaging*. 2007;27(3):154-161. doi:10.1111/J.1475-097X.2007.00731.X
24. Klein T, Magerl W, Hopf HC, Sandkühler J, Treede RD. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. *J Neurosci*. 2004;24(4):964-971. doi:10.1523/JNEUROSCI.1222-03.2004
25. Sandberg M, Lindberg LG, Gerdle B. Peripheral effects of needle stimulation (acupuncture) on skin and muscle blood flow in fibromyalgia. *Eur J Pain*. 2004;8(2):163-171. doi:10.1016/S1090-3801(03)00090-9
26. Kubo K, Iizuka Y, Yajima H, Takayama M, Takakura N. Changes in blood circulation of the tendons and heart rate variability during and after acupuncture. *Med Acupunct*. 2020;32(2):99-107. doi:10.1089/ACU.2019.1397
27. Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev*. 2015;95(2):549-601. doi:10.1152/PHYSREV.00035.2013
28. Morelli C, Castaldi L, Brown SJ, et al. Identification of a population of peripheral sensory neurons that regulates blood pressure. *Cell Rep*. 2021;35(9):109191. doi:10.1016/J.CELREP.2021.109191
29. Pick J. The innervation of the arteries in the upper limb of man. *Anat Rec*. 1958;130(1):103-123. doi:10.1002/AR.1091300109
30. Benito-Martínez E, Senovilla-Herguedas D, de la Torre-Montero JC, Martínez-Beltrán MJ, Reguera-García MM, Alonso-Cortés B. Local and contralateral effects after the application of neuromuscular electrostimulation in lower limbs. *Int J Environ Res Public Health*. 2020;17(23):1-12. doi:10.3390/IJERPH17239028

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Viudes-Sarrión N, Aleixandre-Carrera F, Beltrá P, et al. Blood flow effects of percutaneous peripheral nerve stimulation. A blinded, randomized clinical trial. *Eur J Clin Invest*. 2023;00:e14091. doi:10.1111/eci.14091