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

Running title: Blood flow effects of pPNS

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Abstract

Vasculature is mainly regulated by the autonomic nervous system. However, the sensory-motor nervous system has also been shown to innervate peripheral vessels and modulate vascular tone. Thus, our aim was to investigate the effects of electrical stimulation of a mixed nerve trunk on blood flow in deep arteries and muscle perfusion. Twenty-nine healthy subjects participated in a randomized-crossover and blinded clinical trial. In a randomized order, each participant received a placebo and two different percutaneous peripheral nerve stimulation (pPNS) protocols on the median nerve. Pain Threshold continuous Low Frequency (PT-cLF) consisted in continuous 2Hz high intensity stimulation and Sensory Threshold burst High Frequency (ST-bHF) protocol consisted in five 100Hz bursts with an intensity slightly above detection threshold. Blood flow was assessed bilaterally using Power Doppler Ultrasonography at the main arteries of the arm, and blood perfusion was assessed at the muscles of the forearm, before and after intervention. We quantified blood flow using a semi-automatized software, freely shared here. Regarding results, placebo intervention, consisting only in needle insertion, produced an immediate reduction on peak systolic velocity which affected all arteries, including the contralateral brachial artery, suggesting a generalized effect. Otherwise, the stimulation protocols had no effect on blood flow, with the sole exception of the ST-bHF protocol preventing the peak velocity reduction in the radial artery. In conclusion, vascular tone of deep arteries and intramuscular perfusion are not affected by electrical stimulation of a mixed peripheral nerve trunk, regardless of stimulation intensity and frequency.

Keywords: percutaneous peripheral nerve stimulation, electrical nerve stimulation, Arterial blood flow, Muscle perfusion, Power Doppler Ultrasound, vascular physiology

1 Introduction

The autonomic nervous system regulates blood flow through the release of neurotransmitters acetylcholine and norepinephrine [1]. Additionally, the sensory system has also been shown to innervate and modulate vascular tone through the antidromic release of substance P and calcitonin gene-related peptide (CGRP) [2]. The research about the influence of the sensory system on the vascular tone has been solely focused on perivascular nerves, which follow blood vessels closely toward peripheral organs [1]. Therefore, the vascular consequences of peripheral nerve stimulation are poorly understood.

Percutaneous peripheral nerve stimulation (pPNS) has become popular for pain management in clinical settings [3]. pPNS 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 [3], it is often forgotten that electrical stimulation of a mixed nerve trunk, such as the median nerve can activate the autonomic nervous system, which may alter vascular tone [4]. Additionally, stimulation of sensory afferents may modify peripheral blood flow through reflex arcs and neurogenic vasodilation [2]. In this regard, a recent study found that trigeminal pPNS can increase cerebral blood flow in an animal model of cerebral vasospasm [5]. Moreover, some studies found that transcutaneous electrical nerve stimulation (TENS) affects skin perfusion [6–8]. However, there is a lack of research on the effects of stimulating a peripheral nerve trunk on deep structures such as main arteries or muscles.

Firstly, understanding the effect of peripheral nerve stimulation on deep blood flow would enhance our knowledge of the relationship between the vascular and nervous systems. Secondly, this information has direct clinical implications as it can help identify potential vascular side effects of pPNS applications. This is especially important for patients with conditions like diabetes, peripheral vascular disease [9] or patients at risk of thrombosis, where altered blood flow can increase the risk of thrombogenesis [10]. Therefore, the aim of this work was to investigate the effects of pPNS on blood flow in deep structures. To this end, we analyzed the effects of two protocols over the median nerve on arterial blood flow and muscle perfusion using power Doppler ultrasound in healthy humans. Our hypothesis is that pPNS will increase blood flow in deep structures.

2 Materials and methods

2.1 Study Design

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

2.2 Participants

30 young and healthy subjects (13 females, 23 years old (SD 2.24)) were initially recruited (Figure 1). The exclusion criteria were physically inactive (< 150 min of moderate-intensity activity per week); upper limb pathology; any electrical stimulation and/or puncture contraindication (immunodepression…), taking anticoagulants or pharmacological pain treatment (NSAIDs <24 hours, opioids…), belonephobia, professional athlete or pregnancy.

Participants signed an informed consent in accordance with 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

The electro-stimulator device (EPTE2 BIPOLAR SYSTEM by IONCLINICS SL., Valencia) was used to apply pPNS at the median nerve. A non-beveled, blunt-tipped needle (0.16 × 25 mm, steel material, IONCLINICS SL.) was inserted on the inner upper arm, 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 damage to other structures and optimize electrode positioning. A 5x5 cm surface adhesive electrode placed over the acromioclavicular joint completed the circuit. Subjects were in a comfortable lateral position during the procedure.

Control protocol consisted of needle insertion without current application, even though the electro-stimulator 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 250ms 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 of cases. The *S*ensory *T*hreshold *b*urst *H*igh *F*requency (ST-bHF) stimulation consisted of 5 bursts of 100 Hz stimulation for 5 seconds separated by 55 seconds (summing a

stimulation time of 5 minutes). The PT-cLF was the longest stimulation (16 minutes). We adjusted the other protocols to 16 minutes to maintain the subjects blinding. Specifically, to adjust the ST-bHF protocol to 16 min, the current intensity was 0 during the first 11 minutes of intervention [11] (Figure 2B).

Following intervention, the researcher applied pressure to the needle insertion site for 1 minute to prevent 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. 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 multi-frequency linear transducer (7-14 MHz). The probe position was standardized with a mark on the skin and an angle of insonation <60º. Each measurement lasted ≥ 15 seconds, 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 couldn't be blinded for the 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 3-4 recordings per location averaged and the mean taken from the two most representative recordings. The variables analyzed were: 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 seconds in both arms, in a random 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 color 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 literature [13,14]. Sometimes muscle perfusion analysis is quantitative [12,15], however 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 [16].

The variables extracted were: 1) Area of the color box (cm^2); 2) Number of DS; 3) Relative perfusion area (RPA), percentage of pixels with DS in the color box; and 4) Estimated Fractional Moving Blood Volume (EFMBV), the amount of detectable moving blood [15]. For details on the software and how to use it, see the Supplementary Material (DOI 10.17605/OSF.IO/NVRW8.).

2.5 Sample Size Calculation

Sample size was calculated based on a previous work [17], 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 [18].

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 [11]). 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. Armonk, NY: 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 control protocol effect, we compared pre vs post intervention measurements (paired t-Test or Wilcoxon's Test). To compare between protocols, data was normalized to each day baseline through subtraction (Figure 2C). We analyzed 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 analysis, not preregistered, was performed for additional exploratory analysis with higher statistical power [19].

3 Results

Recruitment ended in November 2020 (started in August). One participant dropped out before allocation due to NSAIDs 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 values are also reported in Tables S1-S2, and all data is available in the OSF repository (Supplementary Material, 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

Our first aim was to assess 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 =0.003, paired t-test, Cohen's d = 0.38), time-averaged medium velocity (TAMEAN) (-1.8 cm·s-1, SD 2.8, p =0.002, d =

0.45) and an increase in resistance index (RI) (0.8 au, SD 2.2, p =0.054, d = 0.39) 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 (Generalized ANCOVA) (Figure 3D-G).

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 =0.001, d = 0.43 and 0.72, respectively) and increased RI (0.09 au, SD 0.14, p =0.001, d = 1.06), similar 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 = 0.018, d = 0.55, ST-bHF vs control). Generalized ANCOVA analysis showed that ST-bHF treatment increased TAMEAN by 22% compared to control (95% CI = [0.15, 4.9] in %, z-score = 2.2, p = 0.025).

There were no significant changes in the ulnar artery except for a non-significant reduction in TAMEAN observed in the PT-cLF group (Table 2). This reduction was non-significant at *post hoc* comparisons (-1.5 cm·s-1, SD 5.1, p = 0.120, d = 0.49, PT-cLF vs control). Our secondary analysis confirmed the difference in TAMEAN between treatments and showed that PT-cLF decreased TAMEAN by 23% compared to control (95% CI = [0.03, 0.43] in %, z-score = 2.3, p = 0.024). In summary, ST-bHF prevented PSV and TAMEAN reduction in the radial artery, while PT-cLF reduced TAMEAN in the ulnar artery, and there were no other significant changes observed in either artery (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 =0.043 and 0.031, d = 0.35 and 0.48, respectively). None of the stimulation protocols effects differed from the ones produced by the control intervention (Figure 3). This suggests that the effect observed in the control group is produced in a generalized way, not only in the territory innervated by the stimulated nerve. Additionally, we have analyzed the time course of the blood flow changes observed after control protocol and found that they were produced immediately after needle insertion (Supplementary Material, DOI 10.17605/OSF.IO/NVRW8.).

3.2 pPNS Effects on Forearm Muscle Perfusion Doppler

We obtained the muscle perfusion signal by using our own software to detect and extract it (Figure 4A-B). The control protocol did not alter the Estimated Fractional Moving Blood Volume (EFMBV), related perfusion area (RPA), or number of Doppler signals (DS) during systole (-0.4%, -0.1% and -0.36%, SD 1.7, 2.2 and 1.9, $p = 0.232$, 0.848 and 0.359, respectively) or diastole (-0.3%, 0.1% and -0.2%, SD 8.7, 1.2 and 1.4, $p = 0.886$, 0.731 and 0.525). However, the stimulation protocols did affect the number of DS (ANOVA, Table 3), but no significant difference was found between specific protocols ($p > 0.05$) (Figure 4C-H).

When the contralateral arm was assessed, the control protocol neither changed EFMBV, RPA and the number of DS detected during the systole (0.2%, -0.3% and 0.3%m SD 2.3, 2 and 2.5, $p = 0.667$, 0.420 and 0.531) and diastole (1.9%, -0.1% and -0.03%, SD 14.6, 1.6 and 1.7, $p = 0.531$, 0.805 and 0.923). In this case, no statistical difference between interventions were found for any variable at systole or diastole (Table 3) confirmed by the secondary analysis (Figure 4I-N). In summary, although a difference was found in the number of DS during the systolic peak in the ipsilateral arm, no variable was strong enough to ascertain a significant difference between two protocols in the muscle perfusion analysis.

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 correctly identified the control, resulting in successful blinding in over 93% of cases. This was surprising as electrical stimulation is difficult to mask. One week passed between interventions, which may have helped subjects forget the previous treatment. We (authors) also tested the protocols on themselves and found that current stimulation was clearly perceived during intervention, but some reported tingling during the control protocol. 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 0.09%. No severe adverse effects were reported.

4 Discussion

In this study, we investigated the effect of pPNS on arterial blood flow and muscle perfusion in the upper limb in young healthy people for the first time. Our results show that the stimulation protocols (ST-bHF and PT-cLF) applied via the median nerve did not have any significant effect on upper limb arterial blood flow or forearm muscle perfusion. 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 lack of vascular effects is surprising. Physiologically, there are several plausible explanations. First, let's discuss the stimulation protocols. 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 [11]. One possibility is that the low intensity of ST-bHF protocol failed to activate high threshold C-fibers, which contribute to vasodilation through antidromic release of CGRP [2,20] and catecholamines release [21]. Another plausible explanation is that TENS currents below motor threshold are unable to increase blood flow [6,22,23]. In healthy individuals, percutaneous stimulation of the common peroneal nerve can increase venous flow to the leg through the contraction of leg muscles. [24].

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 [2]. It is possible that the PT-cLF protocol may have depressed the sensory pathway [25], 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 like a circular argument to us. To explore these possibilities, a high frequency and high intensity protocol should be tested. It is worth noting that other studies have reported no effect of TENS or electro-acupuncture [6,22].

Another possibility for the PT-cLF protocol is that it may be activating blood vessel-related fibers, 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 [21].

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 minutes. This contrasts with reports from other studies that have found an increase in cutaneous blood flow after needle insertion

[26,27]. 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 [28] 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 [29]. In this line, ST-bHF protocol prevented peak systolic velocity reduction in the radial artery, indicating that the effects of peripheral perivascular nerve stimulation on blood flow may depend on the innervation territory [2]. Accordingly, the proximal third of radial artery is partially innervated by the median nerve [30]. Previous studies have also reported increased muscle blood flow when specific dorsal root ganglia are stimulated [2].

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, we could use a probe holder to avoid movement. 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 [6,7,22]. However, we observed no changes in blood flow despite muscle contractions. Replicating this study in patients with peripheral vascular disease or painful conditions would be interesting for future research.

In conclusion, upper limb arterial blood flow and forearm muscle perfusion are unaffected percutaneous electrical stimulation of the median nerve. Physiologically, this evidence suggests that the electrical stimulation of a mixed peripheral nerve trunk at different intensities and frequencies is not able to change the blood flow in deep arteries and muscles of the upper limb. Clinically, pPNS is a safe intervention in terms of secondary effects over the peripheral vascular system.

5 Conflict of Interest

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

6 Author Contributions

MDM and EV envisioned, designed, and coordinated the study. NVS collected all data, contributed to study design and wrote the methods of the first draft of the manuscript. FJO and PB gave clinical advice for the design and implementation of the study, as well as executed the treatments. FJMP gave expert advice regarding Doppler signal acquisition, analysis, and interpretation. FAC and MDM interpreted the results, reviewed the bibliography, and wrote together the first draft of the manuscript, then supervised by EV. FAC also designed and generated the figures. All authors supervised and gave feedback for the last version of the manuscript.

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9 Tables

Table 1. Descriptive Variables of the sample.

Descriptive data (n = 29)			
Quantitative (Mean (SD))		**Qualitative (Frequency)**	
Age	23 (2.4)	Gender (Woman/Man)	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

Table 2. pPNS protocols effects over arterial blood flow variables.

Artery	Variable	Intervention (Δmean (SD))			Repeated Measures ANOVA		
		Control	PT-cLF	ST-bHF	F	P	ηp2
Brachial	**PSV**	-3.4 (5.7)	-2.4 (6.9)	-2.7 (9.4)	0.160	0.853	0.005
	TAMEAN	-2 (2.9)	-1.8 (3.2)	-1.5 (3.8)	0.207	0.814	0.008
	CSA	-.61 (3.2)	-.01 (.03)	-.01 (.03)	0.536	0.592	0.043
	RI	.02 (.09)	.028 (.09)	.027 (.07)	0.099	0.906	0.003
Radial	**PSV**	-3.4 (5.7)	-3.4 (4.8)	-.23 (5.9)	**4.511**	**0.015***	**0.135**
	TAMEAN	-1.9 (2.5)	-2 (2.69	-1.2 (2.3)	1.082	0.347	0.047
	CSA	-.009 (.04)	.0005 (.06)	.0069 (.03)	0.745	0.480	0.029
	RI	092 (.14)	.003 (.16)	.05 (.09)	2.331	0.116	0.143
Ulnar	**PSV**	-2.1 (6.1)	-3.4 (4.9)	-2.1 (7.2)	0.395	0.676	0.013
	TAMEAN	-0.9 (3)	-3 (3.1)	-1.4 (3)	3.089	0.054	0.110
	CSA	-.008 (.04)	.0004 (.04)	.011 (.03)	0.855	0.431	0.033
	RI	0.14 (.13)	.074 (.1.4)	.36 (.09)	1.938	0.153	0.063
Contralateral brachial	**PSV**	-3.1 (7.9)	-1.8 (7.2)	-2.6 (7.7)	0.214	0.808	0.008
	TAMEAN	-1.3 (3.4)	-1.2 (1.9)	-2.7 (2.5)	2.998	0.059	0.107
	CSA	.001 (.03)	.001 (.03)	-.01 (.03)	2.174	0.124	0.080
	RI	.04 (.11)	.01 (.17)	.05 (.06)	0.847	0.434	0.029

PT-cLF: Pain Threshold continuous Low Frequency, ST-bHF: Sensory Threshold burst High Frequency, PSV: Peak Systole Velocity (PSV), TAMEAN: Time-averaged medium velocity, CSA: Cross-sectional Area and RI: Resistance Index. * < 0.05.

Table 3. pPNS protocols effects over forearm muscle perfusion.

Arm	Variable		Intervention (Δmean (SD))			Repeated Measures ANOVA		
			Control	PT-cLF	ST-bHF	F	P	ηp2
Ipsilateral	Size Box		-.17 (.11)	-.11 (.2)	-.16 (.14)	1.065	0.360	0.082
	Systole	EFMBV	.79 (1.6)	.75 (1.2)	.36 (2.6)	0.194	0.825	0.016
		RPA	.23 (2.4)	.95 (2.6)	1.3 (1.9)	0.680	0.516	0.054
		Nº of DS	-0.5 (2)	-1.2 (2)	1.1 (2.7)	**4.378**	**0.024***	**0.267**
	Diastole	EFMBV	2 (5.9)	3.8 (7.4)	-2.9 (14)	1.588	0.225	0.117
		RPA	-.08 (1.5)	-.47 (1)	.34 (1.4)	0.983	0.389	0.076
		Nº of DS	-.19 (1.5)	-.69 (1.7)	.55 (2.5)	1.104	0.348	0.084
Contralateral	Size Box		-.19 (.1)	-.07 (.023)	-.16 (.15)	1.669	0.233	0.233
	Systole	EFMBV	.0001 (3)	.1 (1.4)	-.5 (1.5)	0.306	0.739	0.025
		RPA	.79 (2.2)	.64 (2)	.84 (1.4)	0.061	0.941	0.005
		Nº of DS	.4 (2.8)	.34 (3.4)	.34 (2.5)	0.002	0.967	0.001
	Diastole	EFMBV	-1.7 (12.3)	6.1 (12.5)	7.6 (10.1)	2.508	0.103	0.173
		RPA	.1 (1.9)	.27 (1.1)	.65 (1.2)	0.647	0.532	0.051
		Nº of DS	-.17 (2.1)	.96 (2.7)	.62 (2.2)	1.042	0.368	0.080

PT-cLF: Pain Threshold continuous Low Frequency, ST-bHF: Sensory Threshold burst High Frequency. EFMBV: Estimated Fractional Moving Blood Volume; RPA: Relative Perfusion Area, Nº of DS: Number of doppler signals, * < 0.05.

10 Figures

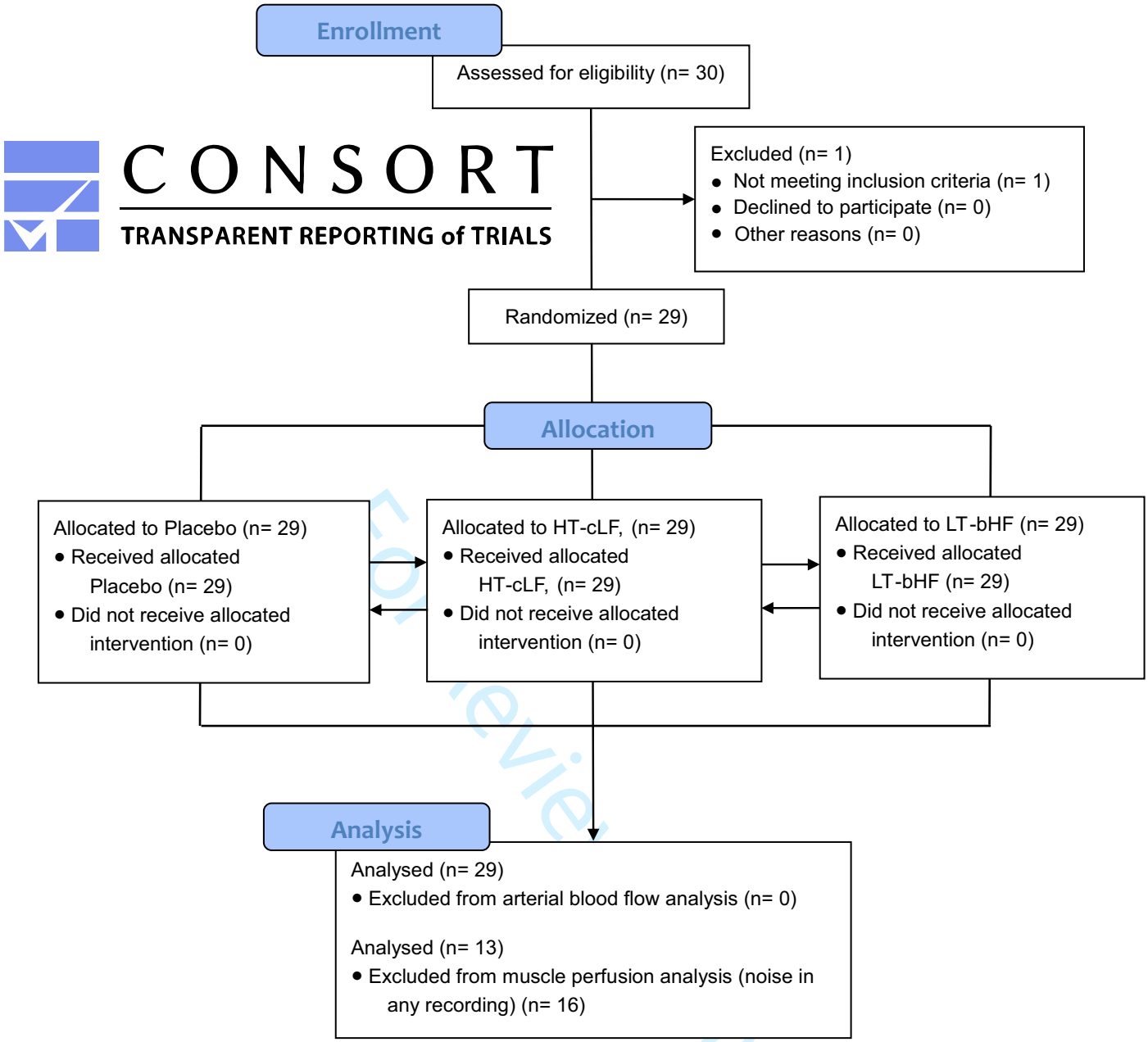
Figure 1. Consort flow diagram for dropouts and sample management. All subjects received all three protocols, and a single dropout was produced prior to assignation due to daily intake of NSAIDs. Thirteen subjects were excluded from muscle perfusion analysis due to noise presence.

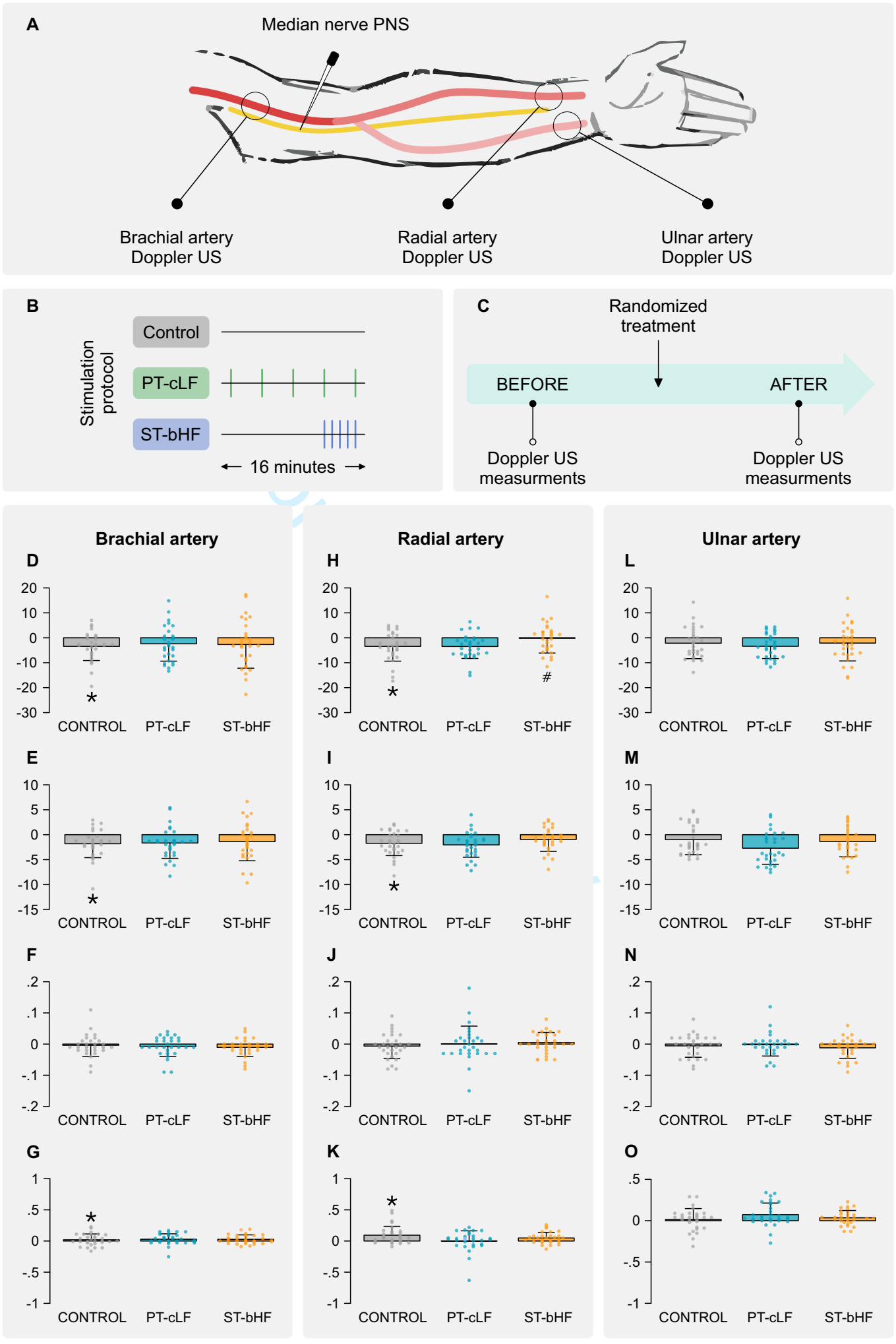
Figure 2. Summary of the experimental design and the effects of median nerve pPNS on ipsilateral arm blood flow. (A) Illustration depicting the placement of the active electrode and the Doppler measurements at brachial, radial, and ulnar arteries. (B) Representation of the protocols used: Control group 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 seconds spaced by 55 seconds, 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 post-treatment mean difference ± SD. * denotes statistical significance between pre- and post-control intervention (Student's paired t-test) while # denotes statistical significance compared to control intervention (rmANOVA test with Bonferroni post-hoc). Statistical significance was considered when p-value < 0.05. 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.

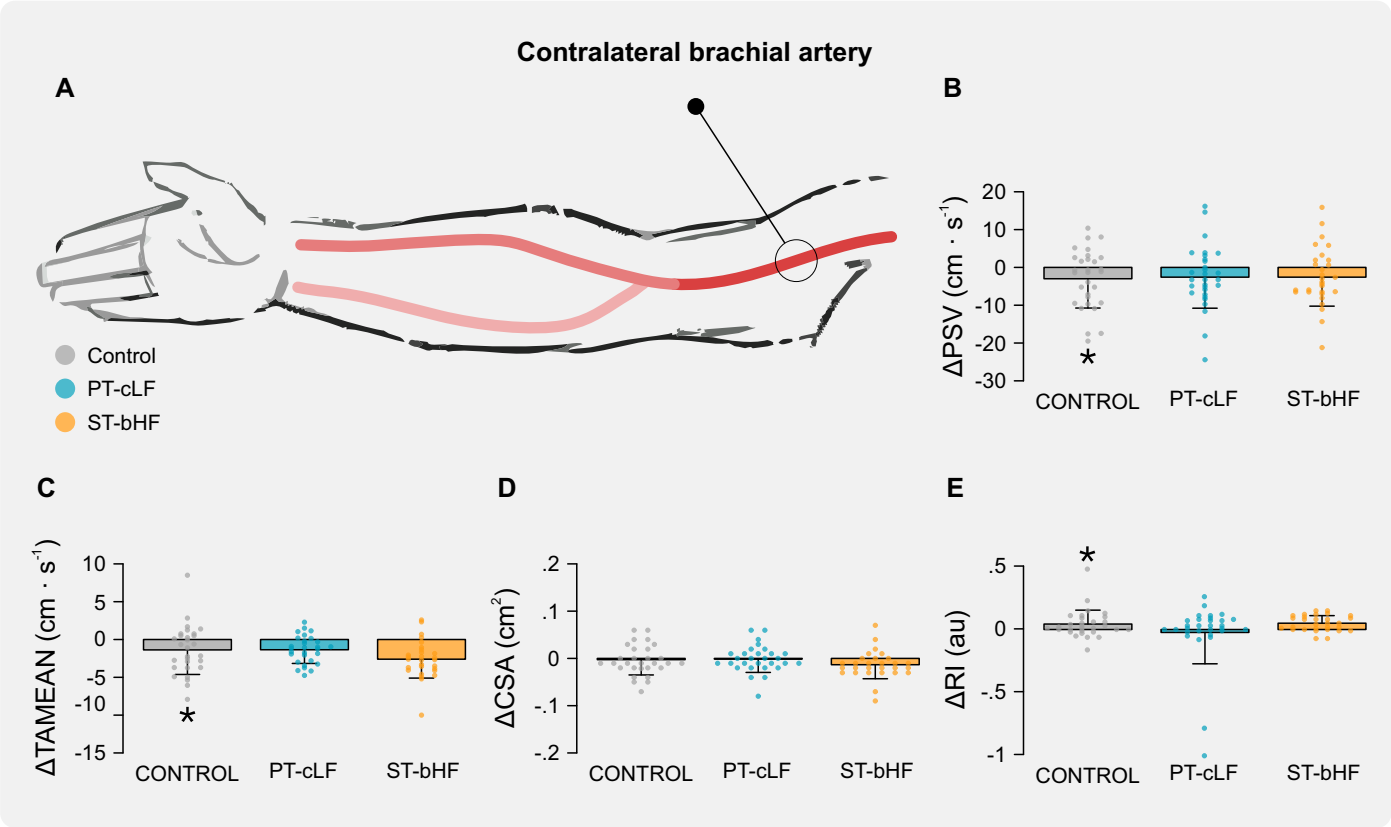
Figure 3. Effects of median nerve pPNS on contralateral arm blood flow. (A) Doppler US was measured at the contralateral brachial artery exclusively. (B) Mean difference of peak systolic velocity (PSV). (C) Mean difference of time-averaged medium velocity (TAMEAN). (D) Mean difference of cross-sectional area (CSA). (E) Mean difference of resistance index (RI). All bars represent post-treatment mean difference ± SD. * denotes statistical significance between pre- and post-control intervention (Student's paired t-test) while # denotes statistical significance compared to control intervention (rmANOVA test with Bonferroni post-hoc). Statistical significance was considered when p-value < 0.05.

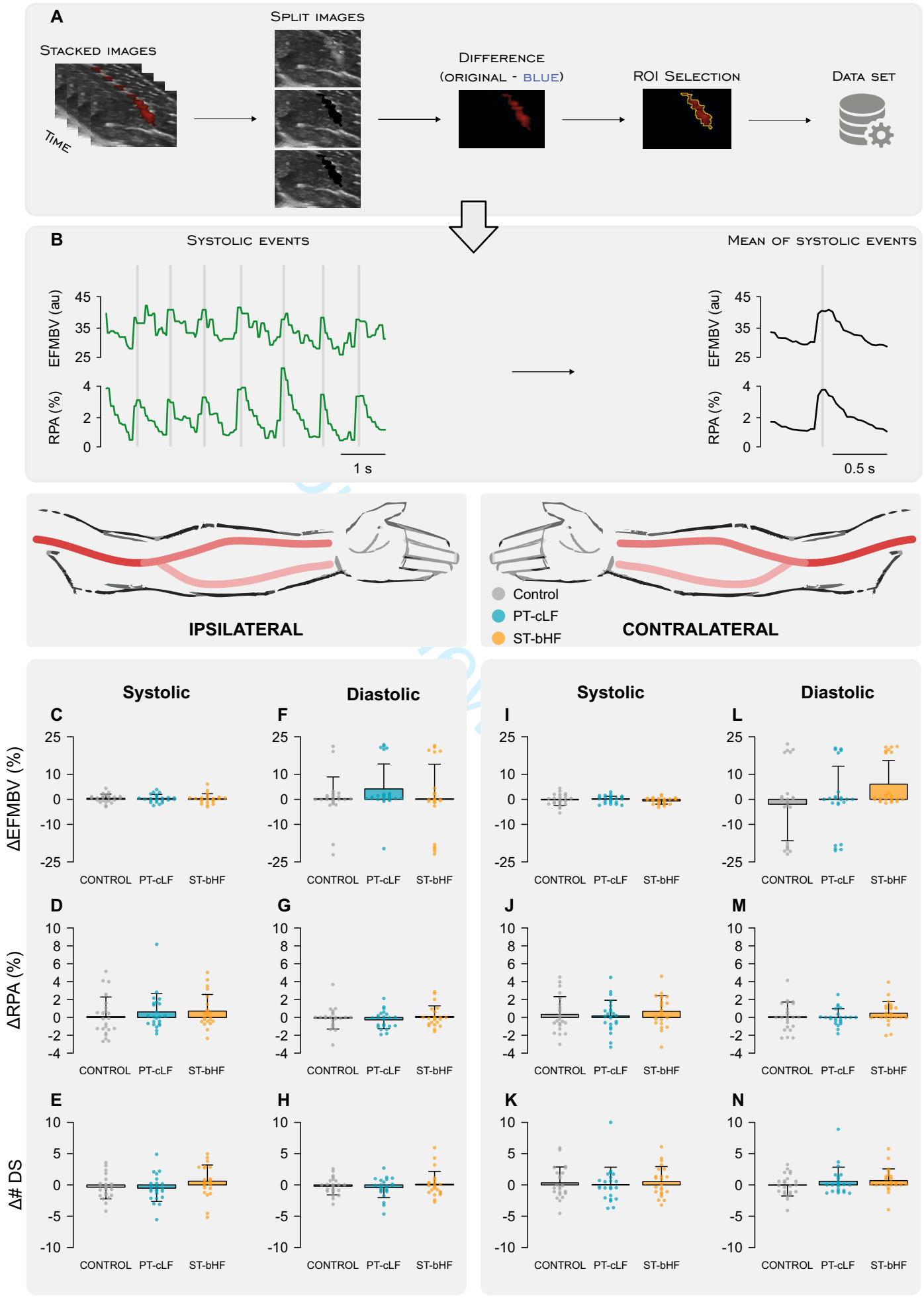
Figure 4. Effects of median nerve pPNS on forearm muscle perfusion. (A) Doppler US images were processed to obtain changes in intramuscular perfusion of the ipsilateral and contralateral forearm. (B) Systolic and diastolic events were detected and averaged using *Spike 2 v8.02*. (C-H) Mean difference 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 post-treatment mean difference ± SD. Statistical significance was considered when p-value < 0.05. For the secondary analysis we used an ANCOVA linear model based on gaussian distribution with Identity link.




A
Median nerve PNS
Brachial artery Doppler US
Radial artery Doppler US
Ulnar artery Doppler US
B
Stimulation protocol
Control
PT-cLF
ST-bHF
16 minutes
C
Randomized treatment
BEFORE
AFTER
Doppler US measurments
Doppler US measurments
Brachial artery
Radial artery
Ulnar artery
D
H
L
E
I
M
F
J
N
G
K
O
ΔPSV (cm · s-1)
ΔTAMEAN (cm · s-1)
ΔCSA (cm2)
ΔRI (au)
CONTROL
PT-cLF
ST-bHF




A
Stacked images
Time
Split images
Difference
(original - blue)
ROI Selection
Data set
B
Systolic events
Mean of systolic events
EFMBV (au)
RPA (%)
1 s
0.5 s
IPSILATERAL
CONTRALATERAL
Control
PT-cLF
ST-bHF
Systolic
Diastolic
ΔEFMBV (%)
ΔRPA (%)
Δ# DS
CONTROL
PT-cLF
ST-bHF