THE EFFECT OF NEUROSTIMULATION ON BLOOD FLOW AND ISCHEMIC PAIN

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by

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PROJECT INFORMATION

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ABSTRACT

THE EFFECT OF TRANSCUTANEOUS NERVE NEUROSTIMULATION ON

BLOOD FLOW AND ISCHEMIC PAIN

Stacey Fishman

Chronic pain caused by lack of blood flow is known as ischemic pain. Neurostimulation, the application of electrical currents through a region of the body, is effective for pain modulation, and it is hypothesized that this can be explained by the gate control theory and alterations of the sympathetic output initiated by the metaboreflex. The decrease of sympathetic output reduces vasoconstriction and improves blood flow. Transcutaneous electrical nerve stimulation (TENS) and interferential currents (IFC) stimulation, both non-invasive neurostimulation techniques, were evaluated for their effects on cutaneous blood flow on the palm. High or low frequency TENS and/or IFC, and the electrode positions (on the forearm or the back) were evaluated. Ischemia was induced to simulate the chronic pain experienced by individuals, and along with pain and blood flow, the amount of time to stabilize blood flow, known as reperfusion time, was investigated. There were no differences from control except for reperfusion time in IFC on the back. This pre-pilot study was limited by sample size, therefore future work with a larger test group will improve the reliability of the data and allow for the evaluation of the effects on blood flow and ischemic pain.

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"What lies behind you and what lies in front of you, pales in comparison to what lies inside of you."

- Ralph Waldo Emerson

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CHAPTER 1: INTRODUCTION

Peripheral artery disease (PAD) is a condition in which there is limited blood flow to the extremities. Many individuals with PAD experience pain even when resting, an advanced condition known as critical limb ischemia. Ischemia occurs when there is insufficient blood flow and cells use anaerobic metabolism to produce energy and perform their normal functions. This process produces lactic acid as a byproduct and aggravates the body's chemoreceptors, initiating pain [1]. Current therapies are targeted toward improving blood flow, but there are few strategies to reduce pain. This study aims to investigate the efficacy of a therapy which utilizes transcutaneous nerve stimulation (TENS) or interferential current (IFC) stimulation in order to increase blood flow in the region of the pain.

1.1 Peripheral Artery Disease and Atherosclerosis

According to the CDC, about 5% of individuals between the ages of 60 and 69 and over 20% of individuals over the age of 80 have PAD [2]. The risk factors for PAD include advanced age, high BMI, smoking, hypertension, and hypercholesteremia. It mainly affects the arteries in the lower extremities, and is caused by atherosclerosis, as seen in **Figure 1** [3]. Intimal thickening is caused by the proliferation of smooth muscle cells, infiltration of macrophages, and the

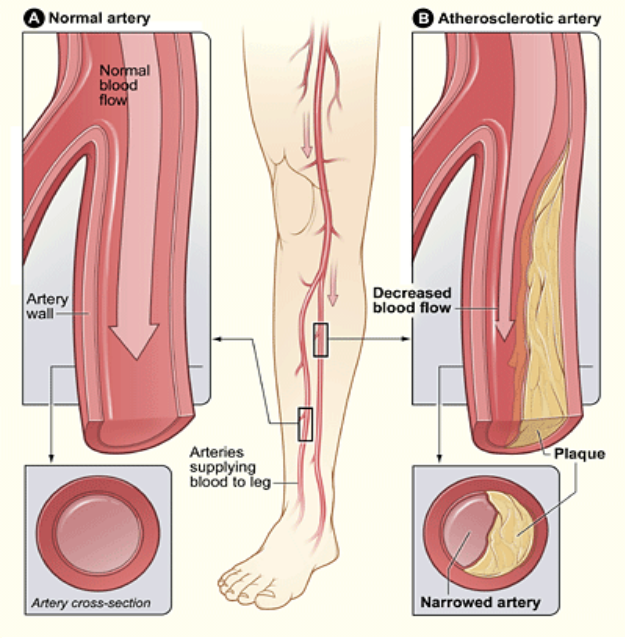

Figure 1. A healthy artery compared to an atherosclerotic artery. The right shows a healthy artery in the lower leg, while the left shows an atherosclerotic artery. The atherosclerotic artery has noticeable plaque buildup and could lead to PAD [5].

build-up of cholesterol [4]. The general mechanisms involved in atherogenesis, the development of atherosclerosis, are illustrated in **Figure 2**. Intimal thickening is activated by the binding of Endothelin-1 (ET-1) to its receptor on smooth muscle cells. This causes the smooth muscle cells to proliferate and migrate to the subendothelium under the action of platelet derived growth factor (PDGF). ET-1 also binds to its receptors on fibroblasts to stimulate proliferation and collagen production. When there are high levels of calcium and cholesterol present, plaques may form [6]. Long streaks of these plaques are known as lesions, and they are used to classify atherosclerosis. Lesions are formed by foam cells, macrophages that metabolize cholesterol, and are deposited in the intimal lining [4]. Macrophages become foam cells when they phagocytose oxidized low density lipoproteins (LDL). LDLs transport triglycerides and cholesterol throughout the body, and when there is an abundance of LDLs in the body, macrophages begin attacking them, using ROS to oxidize them. The oxidized LDLs are then phagocytosed by the macrophages, and the macrophages take on the appearance of being fluffy [6]. The oxidized LDLs are toxic to the macrophages and initiate apoptosis, or programmed cell death, which begins the formation of the necrotic core of the atherosclerotic plaque. Atherosclerosis is enhanced by inflammation. Inflammation occurs due to damage on the vessel lining, and this can be caused by turbulent flow from hypertension. Once there is damage to the vessel wall, endothelial cells, the cells lining vessels, secrete factors which promote a procoagulent state. During the procoagulent state, the lining expresses factors which allow immune cells such as macrophages and T-cells to infiltrate. T-cells produce IL-1, INF-γ, and TNF. All of these factors contribute to inflammation and the procoagulent state, thus continuing atherosclerosis [6]. There are several outcomes of atherosclerotic plaque formation.

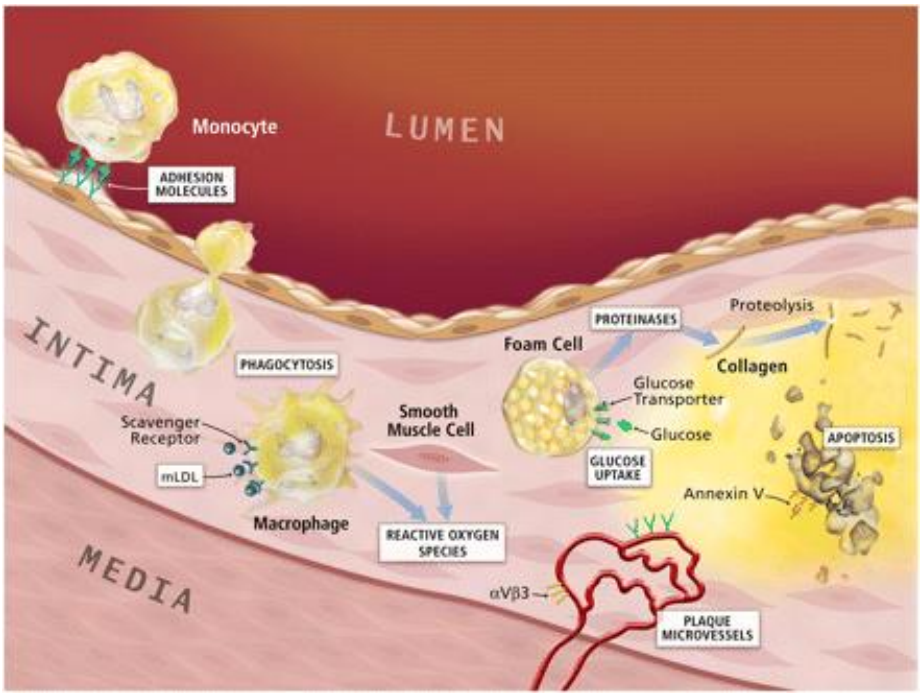

Figure 2. An illustration of the process of atherogenesis. On the left, a monocyte/macrophage is infiltrating the intima of the artery due to the expression of adhesion molecules from inflammation. Foam cell formation can be seen as well as plaque formation due to apoptosis and collagen production around the necrotic core. A bulge in the intima can be seen, known as intimal thickening due to plaque formation [7].

The narrowing of the arteries restricts blood flow and creates flow turbulence. Turbulence in blood flow damages the endothelial lining of the arteries and increases the likelihood of thrombus formation. Thrombi have the possibility of detaching from the surface of the endothelial layer in the artery and embolizing. When a thrombus embolizes, it gets trapped in the smaller vasculature of the body, mainly in the heart causing a cardiac infarction (heart attack), or the brain causing an ischemic stroke.

In addition to thrombus formation, restricted blood flow occurs as a result of the narrowing of the arteries. Less blood flow to limbs such as the lower extremities can cause pain due to a lack of oxygen and can even occur at rest [8]. Ischemic pain occurs when the muscles in the body

begin to use anabolic metabolism, producing lactic acid and lowering the pH of the blood. Cells in the body become damaged from the lack of oxygen and lowered pH and release bradykinin. Bradykinin is released because of its vasodilatory function, but also irritates chemoreceptors, amplifying pain [1].

The majority of treatments for PAD are focused on treating the pain. Several methods include maintaining a healthy diet, exercising, medications to lower blood pressure and cholesterol levels, angioplasty, stenting, and arterial bypass grafting [8]. Many of these treatments have drawbacks. For example, arterial grafting requires an invasive procedure in which a portion of the femoral vein is removed and grafted on the occluded vessel. Grafting has also been performed using a synthetic material such as Dacron, but these grafts have the risk of thrombus formation, as does any foreign material placed in contact with blood. Pain medication is often used to treat PAD.

Pain medication, however, does not treat the underlying cause of the pain, it only masks it. Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin and ibuprofen, are commonly recommended to relieve ischemic pain. NSAIDs work by inhibiting cyclooxygenase-1 and -2 (COX), which normally oxidizes arachidonic acid into prostaglandins, decreasing inflammation. In addition to not improving blood flow, NSAIDs have side effects that can be harmful, such as increased acid production in the stomach (due to reduced prostaglandin levels), causing irritation and ulcers [9]. Therefore, treatments with fewer side effects are preferable.

Neurostimulation has been investigated previously for its effects on reducing pain and improving blood flow and has no known side effects. Many types of neurostimulation have been tested for

this application including spinal cord stimulation (SCS), electroaccupuncture, interferential currents (IFC) stimulation, and transcutaneous electrical nerve stimulation (TENS).

1.2 Neurostimulation Techniques

SCS, which is performed by inserting epidural needles into the spinal cord, increases peripheral blood flow. More specifically, a lead containing between 4 to 32 electrodes is passed through the inner portion of the needle into the midline of the spinal column [10, 11]. This approach can increase blood flow in the foot as well as reduce ischemic pain in the hands and feet following SCS treatment [10]. It has been hypothesized that SCS deactivates sympathetic tone and thus decreases pain and increases blood flow [12, 13]. The deactivation of the sympathetic nervous system reduces pain by the inhibition of nocioceptors, thus interrupting the pain signal. Although SCS appears to be a promising treatment for PAD (reducing pain and increasing blood flow), there are some limitations. This treatment requires a skilled physician or surgeon to implant the SCS system, and is not ideal for our research because it involves performing a surgery on healthy college-aged students, which would be above our capabilities.

A less invasive method of neurostimulation is electroaccupuncture. Electroacupuncture is performed similarly to acupuncture; needles are inserted at specific acupoints on the body, which will give a desired effect, but the needles in this case are attached to electrodes. Acupuncture has been historically used for pain management, so it could be potentially used to treat ischemic pain. Its cardiovascular effects have been researched in many animal models, but less so in humans. Depending on the location of the treatment, electroaccupuncture can increase blood flow in areas of ischemia [14]. In addition, electroaccupuncture may cause the release of endogenous opioids and monoamines to mitigate pain and NO to dilate vessels [15]. This

method of neurostimulation appears to be promising, but it requires a licensed acupuncturist to properly place the needles/electrodes.

Two non-invasive methods of stimulation are IFC and TENS. IFC is thought to function by causing deeper stimulation to the tissue, therefore affecting a greater number of afferent neurons. It uses a biphasic pulse which is modulated between 4100 Hz and 4000 Hz, creating a unique current illustrated in **Figure 3**. The interference between the two frequencies creates a modulated frequency of 100 Hz applied to the tissue [16]. The crisscrossed electrodes allow for the currents to travel through the length of the tissue, penetrating the tissue further and thus stimulating more neurons. TENS on the other hand produces a current that travels horizontally and provides more of a superficial stimulation. TENS also produces a biphasic current and functions by stimulating the afferent neurons, similar to the current seen in channels 1 or 2 of **Figure 3**. Interestingly, these two methods of stimulation may relieve pain through differing mechanisms [16]. These two types of stimulation will be investigated in this study because they are non-invasive and have been proven effective at reducing ischemic pain and improving blood flow in other studies.

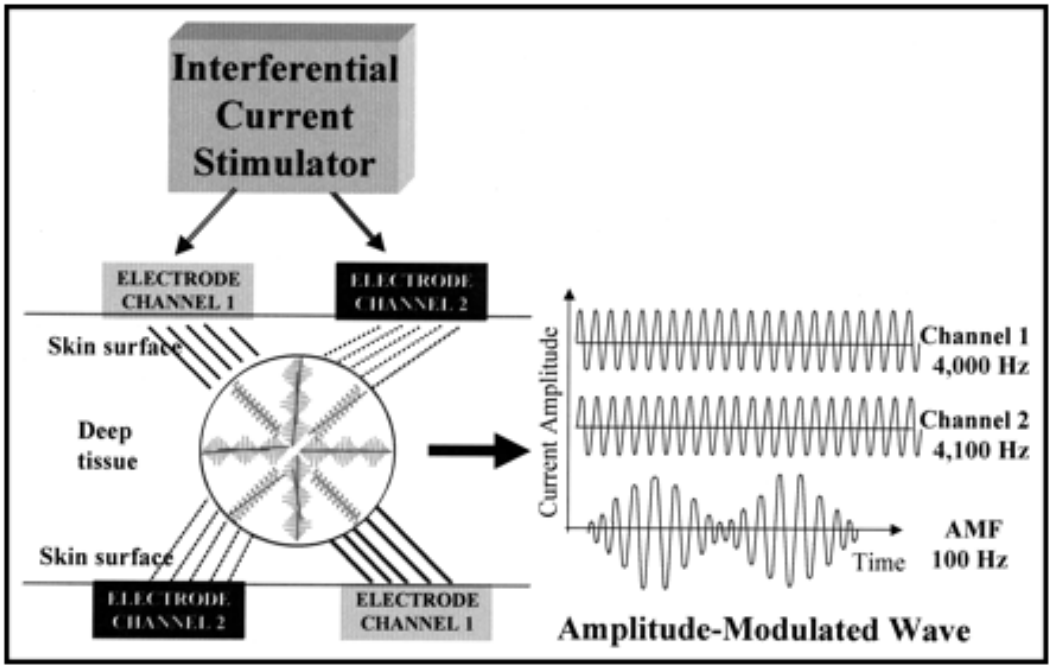

Figure 3. An illustration of the modulated frequency produced by IFC. The interference in Channels 1 and 2 produces a modulated frequency depicted in the AMF chart [16].

1.3 Mechanism

There are several hypotheses to explain how pain signals are received and sent throughout the body. The most widely supported is the gate control theory. The gate control theory was first hypothesized in 1965 and stated that small neuron fibers, C fibers, transmit pain signals while large diameter fibers, labelled Aβ, transmit tactile sensations. The gate control theory suggests that only one of these types of fibers can transmit signals while the other is inhibited by a gate. As seen in **Figure 4**, these fibers transmit nociceptive information via action potentials to projection cells (SG) which are transmitted to the brain or inhibitory neurons in the spinal cord [17].

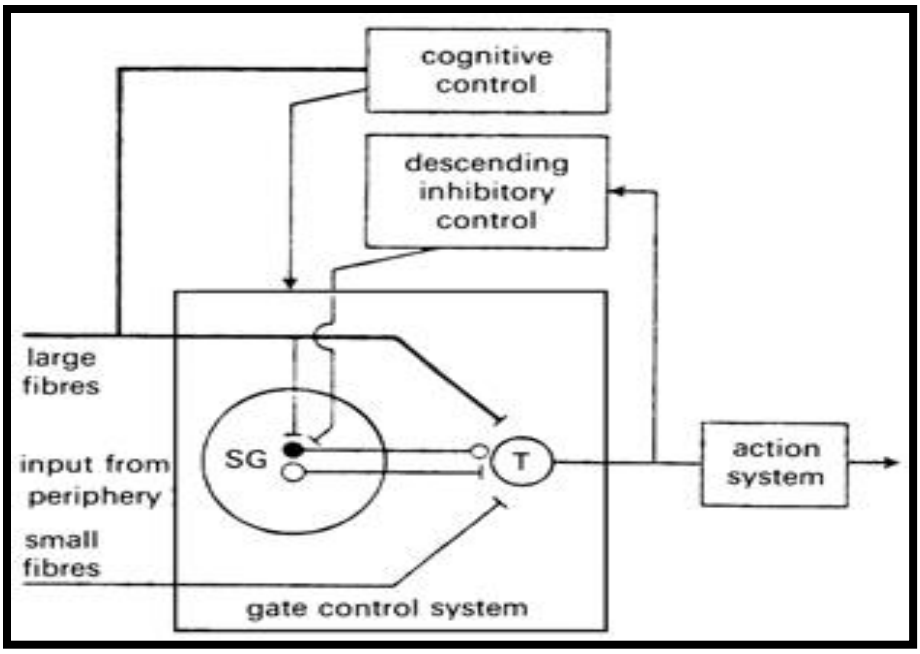

Figure 4. The gate control theory. Large and small diameter fibers cannot transmit signals at the same time to projection cells in the spinal cord. This is possible through the gate system illustrated in this figure [17].

In addition to the gate control theory, the metaboreflex may be activated during ischemic conditions. The metaboreflex is triggered by cardiovascular exercise or ischemic byproducts such as lactic acid, which stimulate the small afferent fibers to increase sympathetic output [18]. The increased sympathetic output increases heart rate and causes vasoconstriction to elevate blood pressure. In healthy individuals, this reflex occurs to improve oxygen delivery during exercise, but in individuals experiencing ischemia, this reflex is constantly activated. Therefore, there is reduced waste removal from these active cells and no functional sympatholysis. This causes increased sympathetic output, seen in increased catecholamine levels, which cause increased heart rate and vasoconstriction.

In order to evaluate this proposed mechanism of the metaboreflex, we measured heart rate, systolic (SBP) and diastolic (DBP) blood pressures, perfusion, and ischemic pain, and calculated

vascular resistance (CVR) and mean arterial pressure, while activating the metaboreflex and inducing ischemia. We hypothesize that application of TENS or IFC at the dermatome C7 and T4 on the back, or the application of TENS or IFC on the forearm will decrease sympathetic activity. The C7 dermatome innervates the arms and the T4 innervates the heart. The decrease in sympathetic output will be demonstrated by increased blood perfusion, decreased heart rate, decreased vascular resistance, and decreased blood pressure and MAP in induced-ischemic individuals compared to healthy individuals. Additionally, we hypothesize that a decrease in sympathetic activity will occur in individuals who receive TENS or IFC compared to no treatment (control) as indicated in **Table I** when experiencing post-exercise circulatory occlusion (PECO).

Table I – Expected Outcomes from the Application of TENS in the Pilot Study

	Placebo		**TENS**	
Expected Outcome	PECO-	PECO+	PECO-	PECO+
Heart Rate	↓	-	↓	-
Blood Pressure	↓	-	↓	↓↓
Blood Flow	↓	-	↑	↑↑
Vascular Resistance	↓	-	↓	↓↓
Mean Arterial Pressure	↓	-	↓	↓↓
Ischemic Pain	-	-	-	↓↓

CHAPTER 2: METHODS OF PRE-PILOT WORK

2.1 Test Subjects

Two healthy college students, both 23 years old, were observed in this study. They had no known illnesses.

2.2 Experimental Design

To investigate the effects IFC and TENS on blood perfusion and ischemic pain during induced ischemia, we evaluated varying frequencies and locations of stimulation, based on parameters from previous studies [19, 20, 21]. The modalities investigated were TENS at 100 Hz and 4 Hz, and IFC at 100 Hz applied to the forearm or the back. **Table II** indicates the optimized treatment settings used in this study. Each individual completed the control and treatment protocols approximately every other day, performing the control protocol first with a 30-minute break in between testing periods to prevent residual effects of neurostimulation.

Table II – Treatment Parameters for TENS and IFC Testing

Treatment Method	Frequency (Hz)	Pulse Width (μs)	Treatment Duration (min)	Intensity Range (mA)
TENS Back– High Frequency	100	200	15	12-13
TENS Forearm – High Frequency	100	200	15	6-8
TENS Back – Low Frequency	4	200	15	11-14
TENS Forearm – Low Frequency	4	200	15	6-8
IFC Back	100 (modulated)	200	15	7-11
IFC Forearm	100 (modulated)	200	15	5

A data acquisition system (Powerlab 26T, ADInstrument) and digital recording software (LabChart 7 Pro, ADInstruments) were used to collect blood perfusion data. **Appendix A** details the measurement tools used to collect respiration, grip force, and blood perfusion information through the data acquisition system.

2.3 Protocol

Blood perfusion, blood pressure, and heart rate were measured using a laser Doppler system (VMS-LDF2, Moor Instruments) and an automated sphygmomanometer (Life Source, Model #UA-767 Plus) under similar conditions described in previous studies [22]. **Figure 5** illustrates the configuration of the testing equipment. Briefly, the volunteer sat up straight in the chair with their forearms resting on the table in a supine position, and for more comfort and fewer motion artifacts, a pillow was available for the forearms to rest on. Next, the manual

sphygmomanometer (CVS, Model #BPAG1-20CVS) was placed the left forearm, about 3 inches distal to the elbow. The automated sphygmomanometer was placed on the upper right arm. The laser Doppler probes were attached on the lower surface of the palm, 2 cm distal from the wrist or just proximal to the antecubital fossa. Finally, the neurostimulation (inTENSity Select Combo) probes were placed on the C7 and T4 dermatomes indicated in **Figure 6A,** or the forearm, **Figure 6B**.

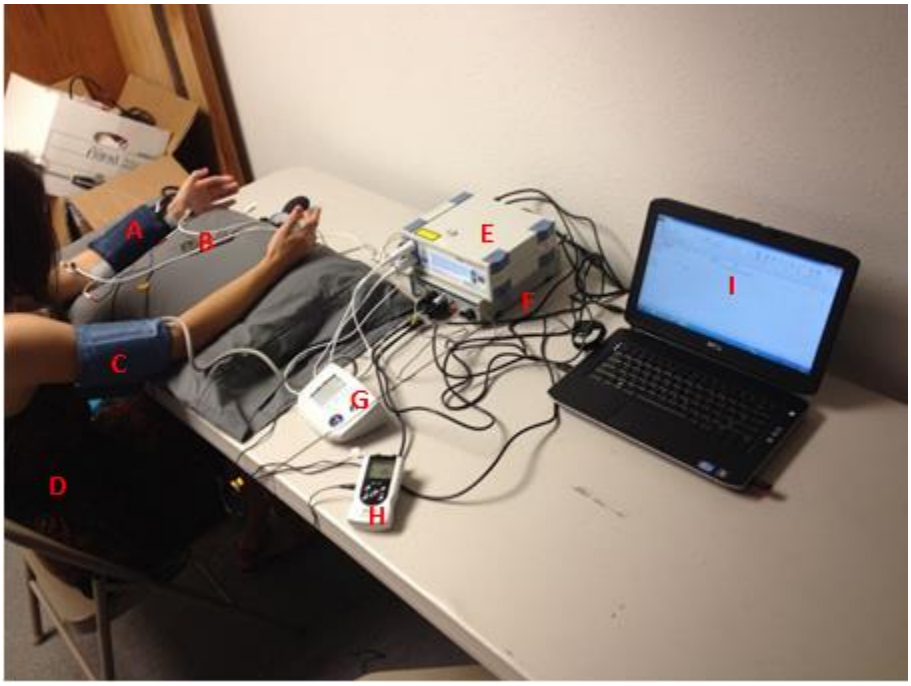

Figure 5. Experimental set-up for pre-pilot work. A) Manual blood pressure cuff B) Hand dynamometer C) Digital blood pressure cuff D) Respiration belt E) LDF system F) PowerLab system G) Blood pressure monitor H) Stimulator I) Laptop with LabChart software.

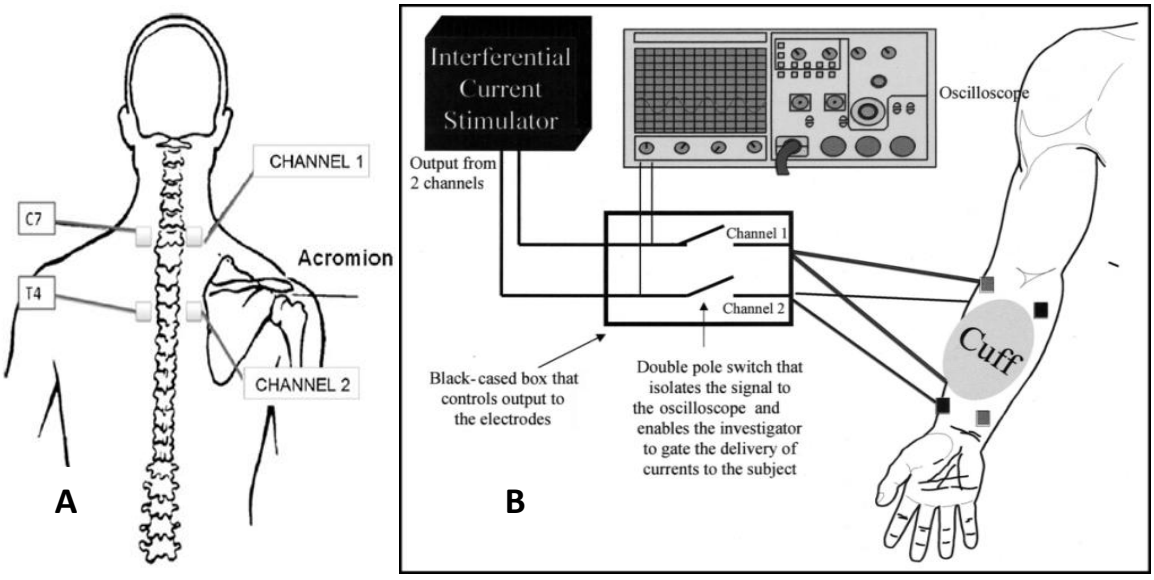

Figure 6. The placement of neurostimulation electrodes. (A) Location of electrode placement on the back, C7 and T4 dermatomes. (B) Electrode placement for neurostimulation on the forearm for IFC, for TENS probes placed with Channel 1 having both probes proximal and with Channel 2 both probes distal, with the polarities the same located on the same side between channels [21].

Once the individual was properly instrumented, they performed hand-grip contractions using the hand dynamometer for 3 repetitions of 2 seconds each, which were averaged to determine the maximum voluntary contraction. Twenty-five percent of the max contraction force was calculated. Next the individual experienced 3 minutes each of pre-stimulation, baseline, exercise, occlusion, and recovery, **Figure 7**. Pre-stimulation occurred before all other steps, and during pre-stimulation, the individual only received the neurostimulation; no measurements were taken. Skin temperature was recorded at the beginning of testing via the Moor skin probes. Blood pressure was measured at the middle of the baseline, exercise, occlusion, and recovery portions, followed by the ischemic pain level. Ischemic pain was measured via the Numerical Rating Scale (NRS).

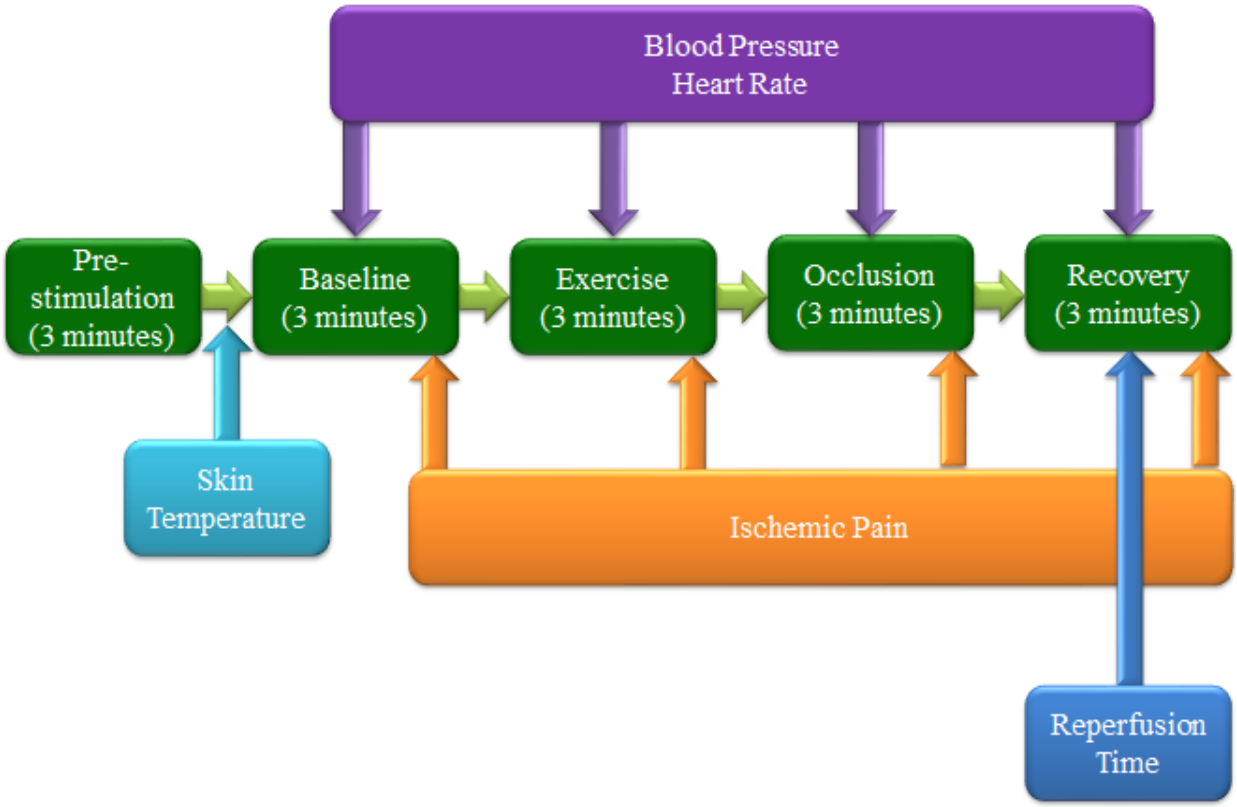

Figure 7. A visual progression of the steps performed in testing procedure. The arrow indicates at what time during that phase the measurement was taken. If running a control experiment, no stimulation was administered starting at the beginning of the pre-stimulation step. Each step had a fixed duration of 3 minutes.

During pre-stimulation, the individual began receiving neurostimulation and the intensity of the stimulation was set to just below the motor threshold and also to an intensity that did not cause discomfort. During control testing, pre-stimulation was simulated but not administered. During pre-stimulation and baseline, the individual sat still and did not talk, but at the beginning of exercise, the individual applied 25% of his/her maximum contraction force to the hand grip dynamometer for 3 minutes. After 3 minutes of exercise, the dynamometer was removed from the hand and the manual sphygmomanometer was inflated to 180 mmHg to induce ischemia for 3 minutes. The sphygmomanometer was deflated to allow reactive hyperemia and recovery. During this pre-pilot work, control data was recorded first, followed by the treatment; a

representative data trace can be seen in **Figure 8**. To normalize the data and reduce individual variation, the data was presented as the percent changes from baseline. Two-way ANOVA followed by Tukey post hoc test analyses were performed.

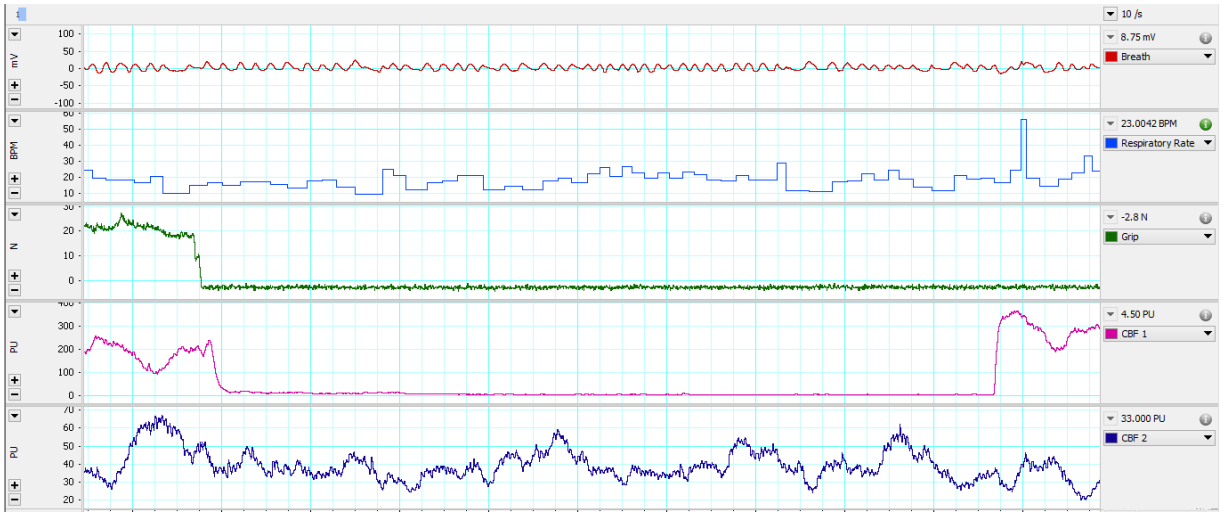

Figure 8. A representative data trace acquired during testing. The top red line corresponds to breathing in mV, the light blue line is breathing rate in breaths per minute (BPM), the green line is grip force in N, The pink line is distal blood perfusion, and the blue line is proximal blood perfusion. The trace is showing a time of occlusion (minimal distal blood flow).

CHAPTER 3: RESULTS

3.1 TENS at 100 Hz on the Back

To test the hypothesis that neurostimulation will improve blood perfusion in healthy individuals with induced ischemia, high frequency TENS was applied to the back, but there were no significant changes compared to control, **Figure 9**. However, the palm perfusion tended to increase during recovery while the brachial perfusion remained stable during all phases. The SBP tended to increase, while the DBP deviated from the results seen in the control. Heart rate showed an inverse pattern from the control, while the MAP in the treatment varied due to the inconsistent blood pressure results. The CVR in the palm tended to decrease in the treatment and increase in the brachial region compared to control.

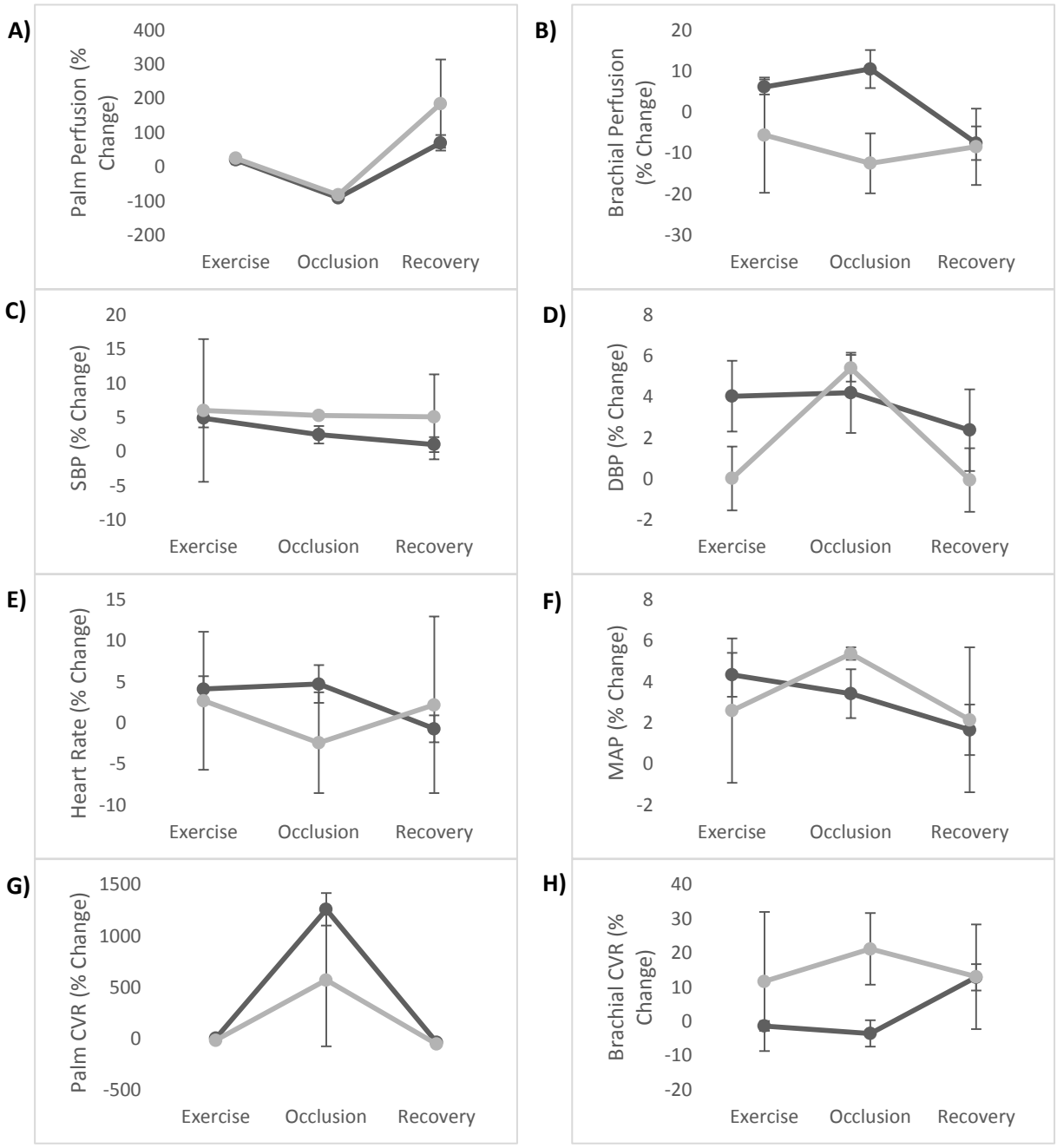

Figure 9. Hemodynamic responses to TENS application on the back at 100 Hz (Treatment) as compared to Control. Treatment in light gray compared to control in dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.2 TENS at 100 Hz on the Forearm

The next neurostimulation tested was high frequency TENS applied to the forearm. There were not any significant differences found, but some similar trends to TENS on the back at 100 Hz were observed, **Figure 10**. These similar trends included increased palm and brachial perfusion and decreased vascular resistance compared to control. Heart rate was higher in this treatment than control. Like TENS at 100 Hz on the back, the treatment palm CVR was lower, but the brachial CVR was also lower all compared to control.

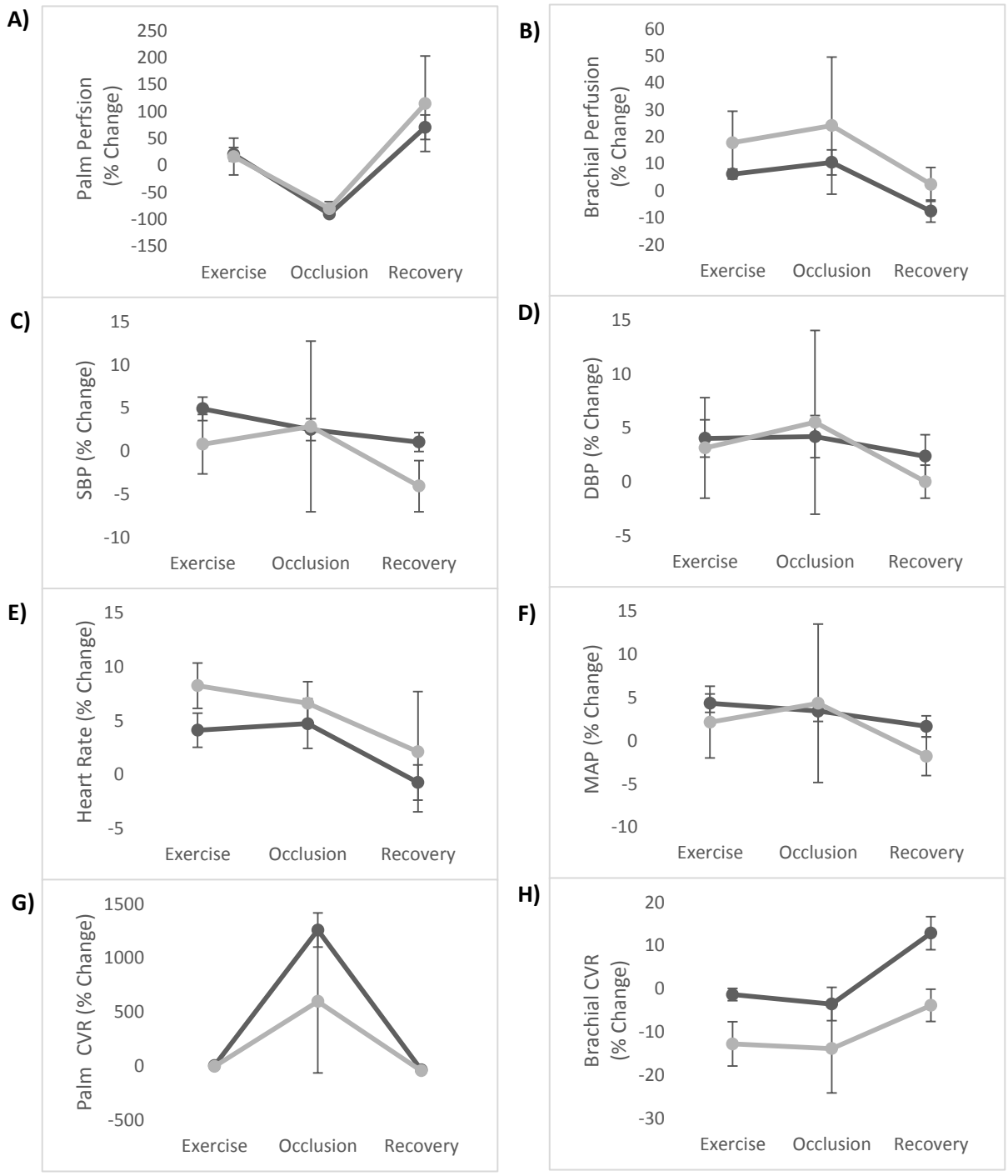

Figure 10. Hemodynamic responses to TENS application on the forearm at 100 Hz (Treatment) as compared to control. Treatment is light gray and control is dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.3 TENS at 4 Hz on the Back

When low frequency TENS was applied to the back, no significant changes between control and treatment groups were observed, **Figure 11**. With this specific treatment, perfusion on the palm as well as in the brachial region tended to increase compared to control, meaning there was improved blood flow. Like the previous treatment groups, a correlation between SBP, DBP, and MAP could not be made. Heart rate also did not appear to have a noticeable trend. The palm and brachial CVRs were both lower than the control.

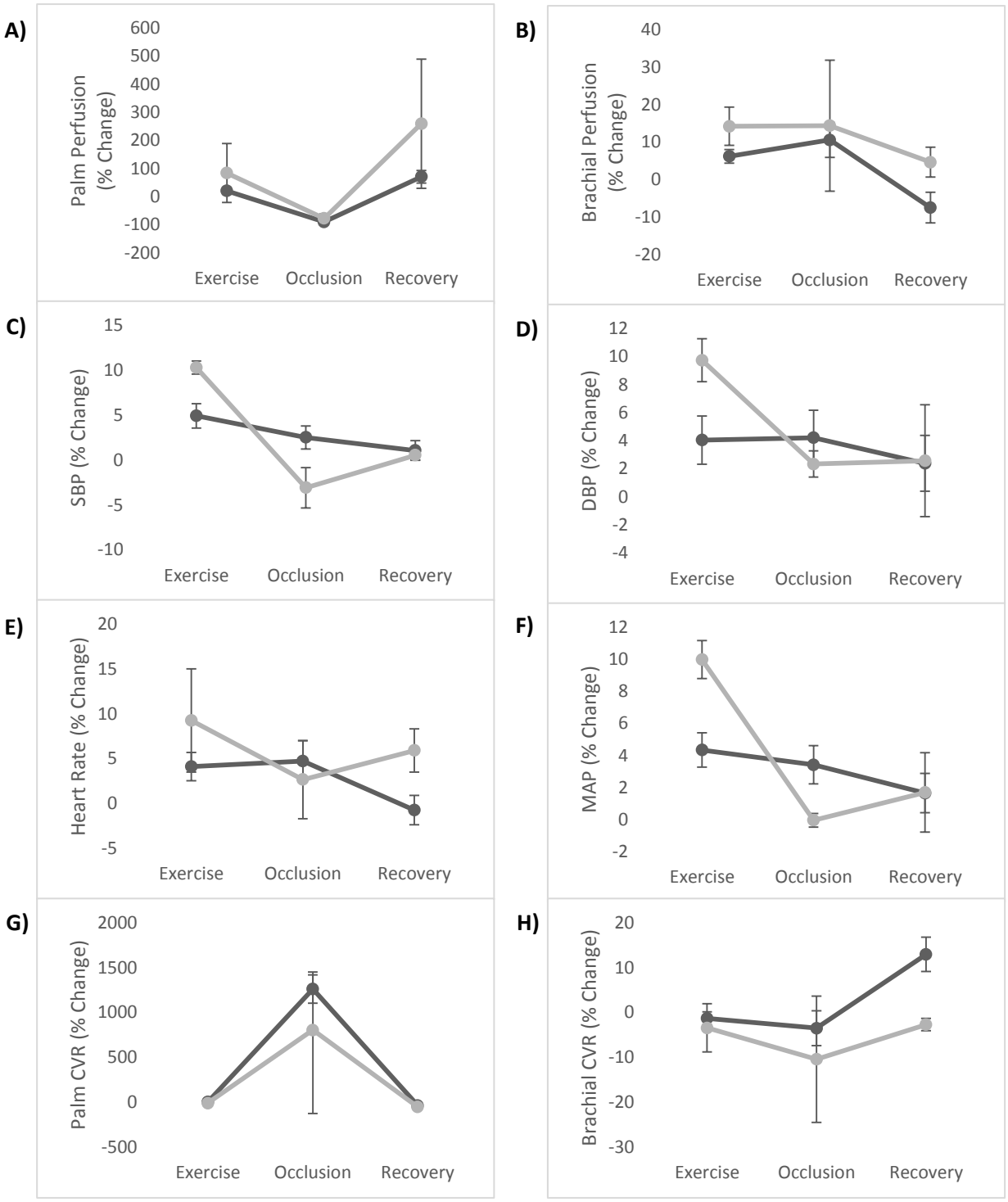

Figure 11. Hemodynamic responses to TENS application on the back at 4 Hz (Treatment) as compared to control. Treatment is in light gray, control in dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.4 TENS at 4 Hz on the Forearm

Investigating low frequency TENS on the forearm did not present any significant differences from control data, **Figure 12**. The palm perfusion was again very similar to control data, while brachial perfusion was tended to increase in the treatment group. The treatment presented lower SBP, while the DBP was very similar to control. For this reason, the MAP decreased more than the control. The heart rate remained lower in the treatment group than control as well as brachial CVR, while palm CVR was almost indistinguishable from the control.

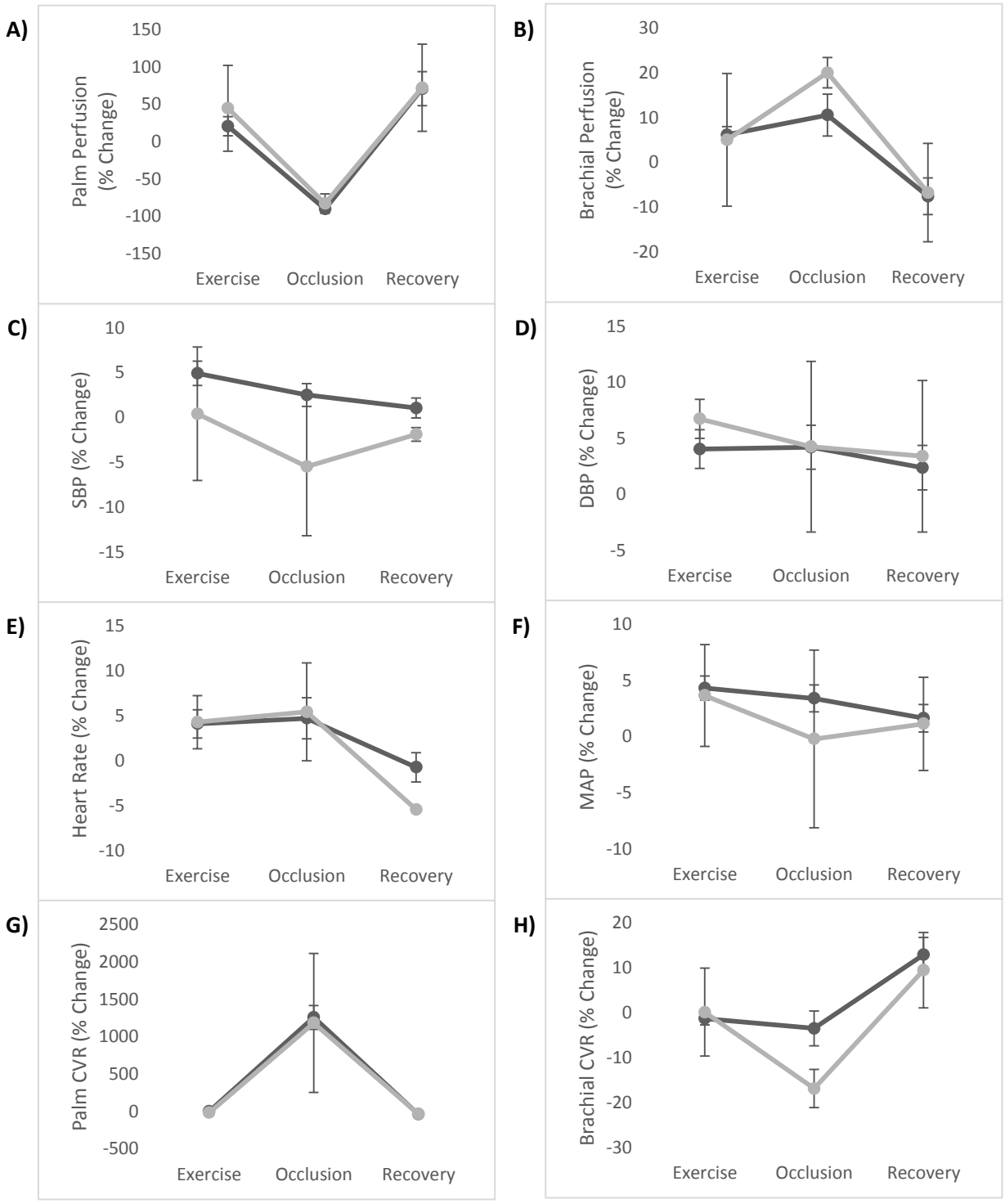

Figure 12. Hemodynamic responses to TENS application on the forearm at 4 Hz (Treatment) as compared to control. Treatment is represented in light gray, control in dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.5 IFC on the Back

After all the combination of frequencies and stimulation locations for TENS were investigated, the application of IFC on the back was compared to control, **Figure 13**. There were no significant differences, but the palm perfusion tended to decrease compared to control. No trend could be seen in brachial perfusion, and similarly there was no trend seen in SBP, MAP, or brachial CVR. DBP was very similar to control data, and HR in the treatment was higher than control. CVR in the palm was lower than control which has been seen in several other treatment groups.

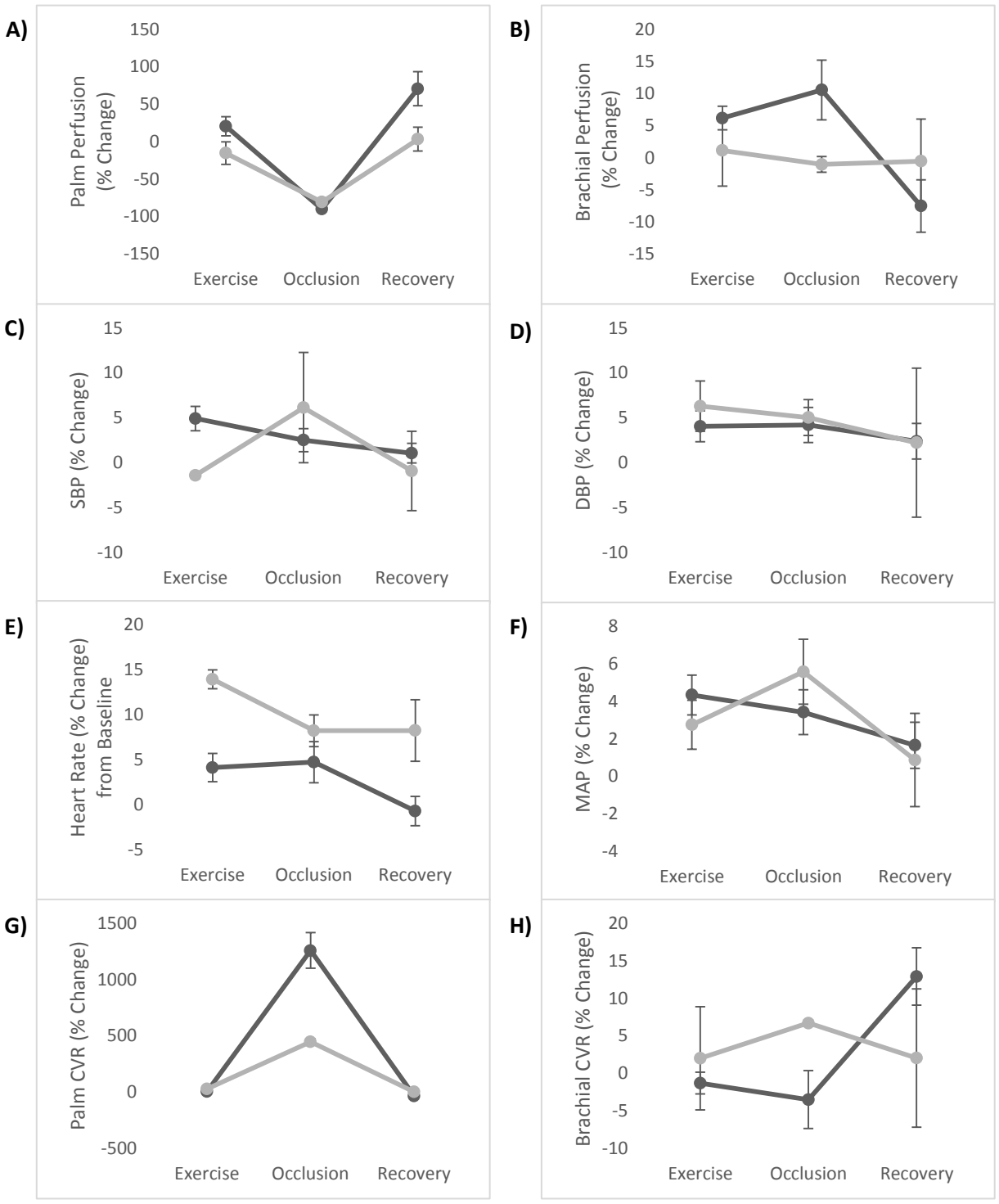

Figure 13. Hemodynamic responses to IFC application on the back (Treatment) as compared to control. Treatment is represented in light gray and control in dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.6 IFC on the Forearm

The results from comparing the percent change in IFC applied to the forearm to control are displayed in **Figure 14**. There were no significant differences between the treatment and the control. For this treatment, the percent change in palm perfusion, heart rate, and CVR in the palm are virtually identical to the control data. Perfusion in the brachial region tended to decrease compared to the control. No correlation could be made for the SBP, and for all the other measured parameters, the treatment had lower values than control (DBP, MAP, and brachial CVR).

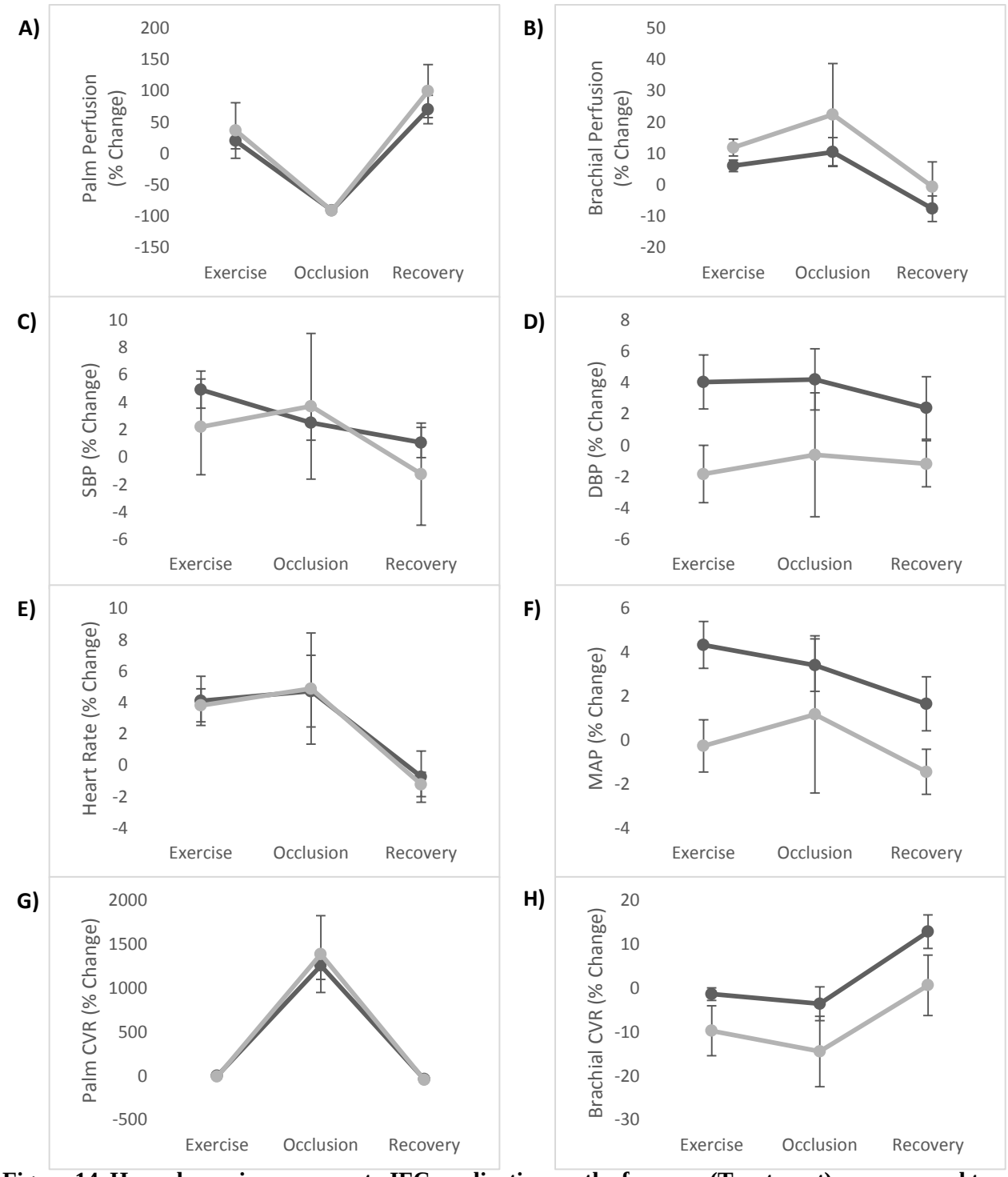

Figure 14. Hemodynamic responses to IFC application on the forearm (Treatment) as compared to control. Treatment is represented in light gray, control in dark gray. A) Blood flow in the left palm; B) Blood flow in the left anterior brachial region; C) Systolic blood pressure D) Diastolic blood pressure E) Heart rate F) Mean arterial pressure G) Cutaneous vascular resistance in the palm; H) Cutaneous vascular resistance in the anterior brachial region.

3.7 Combined Data Analysis

The percent changes from baseline were compiled for each testing parameter, blood perfusion, heart rate, blood pressure, etc., to more effectively compare the impact of each type of stimulation compared to control; error bars were excluded from these plots for enhanced figure visualization. The first parameter compared was palm blood perfusion, **Figure 15**. It was expected that the application of neurostimulation would increase this compared to the control. In this figure, control data was represented with the blue line. There were no significant changes observed, but there was a trend for increased perfusion in exercise, decreased in occlusion, and a large increase in recovery.

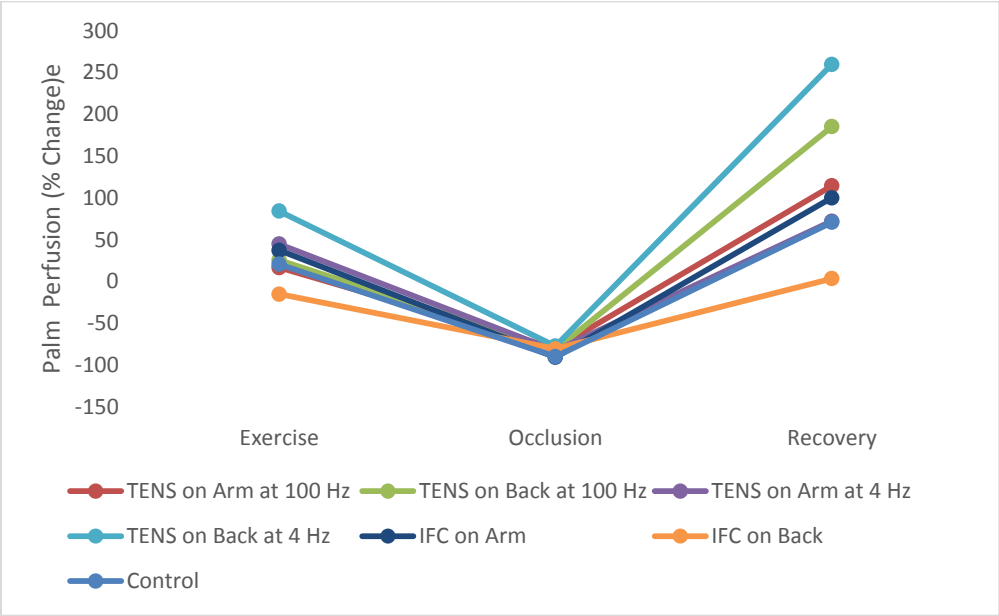

Figure 15. The percent change in palm blood perfusion in each treatment method and control. The percent change in palm blood perfusion in each treatment method and control TENS at 4 Hz on the back had the greatest increase in perfusion, while all others showed improved perfusion over the control except for IFC on the back.

The next parameter investigated was brachial blood perfusion, **Figure 16**. Brachial perfusion was expected to increase with the application of neurostimulation during all testing phases compared to control. Through testing, the trend of the data confirmed this except in IFC on the back and high frequency TENS on the back.

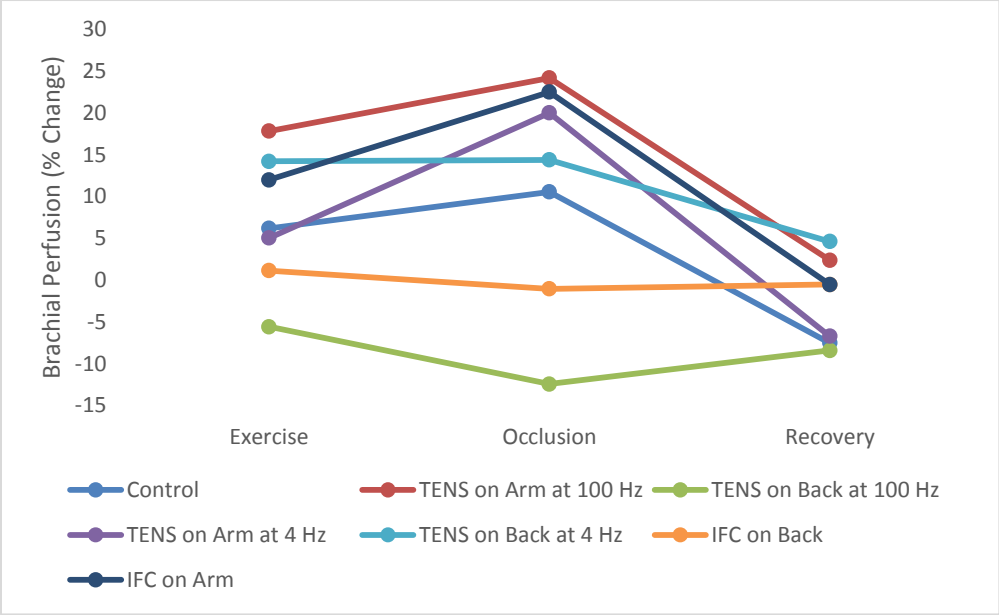

Figure 16. Percent change in brachial blood perfusion for each treatment method. All treatments experienced improved perfusion compared to the control, the highest of which was TENS on the arm at 100 Hz, excluding IFC on the back and TENS on the back at 100 Hz.

Another important indicator of vasodilation is blood pressure. SBP changes were compared between all treatment groups in **Figure 17**. It was observed that the SBP generally increased during exercise, decreased during occlusion, and increased during recovery. It was predicted that there would be a decrease in systolic blood pressure compared to control, but the mixed results are not able to justify this hypothesis.

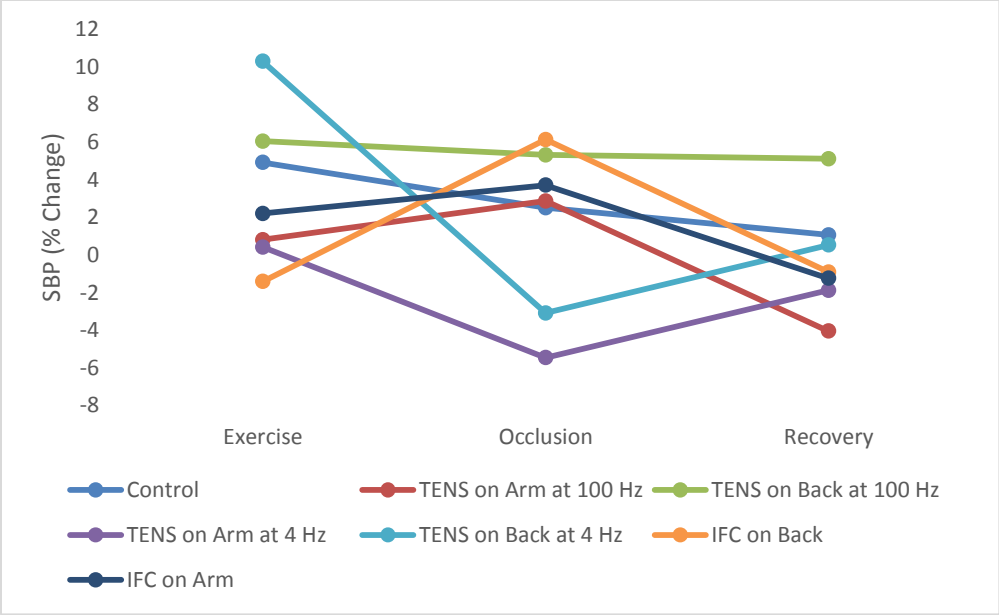

Figure 17. Percent change in SBP based on treatment method. Varying trends in the results were observed, with treatments presenting different changes in SBP depending on the testing step. For example, TENS on the back at 4 Hz has a v-shaped response, while the control group almost has a linear change. TENS on the back at 100 Hz presented a similar pattern to the control group and had a higher percent change. TENS on the arm at 4 Hz however the greatest decrease in SBP compared to control.

The DBP showed similarly inconclusive data, **Figure 18**. It was expected that DBP would also decrease during all testing phases compared to control with the application of neurostimulation, but this could not be confirmed.

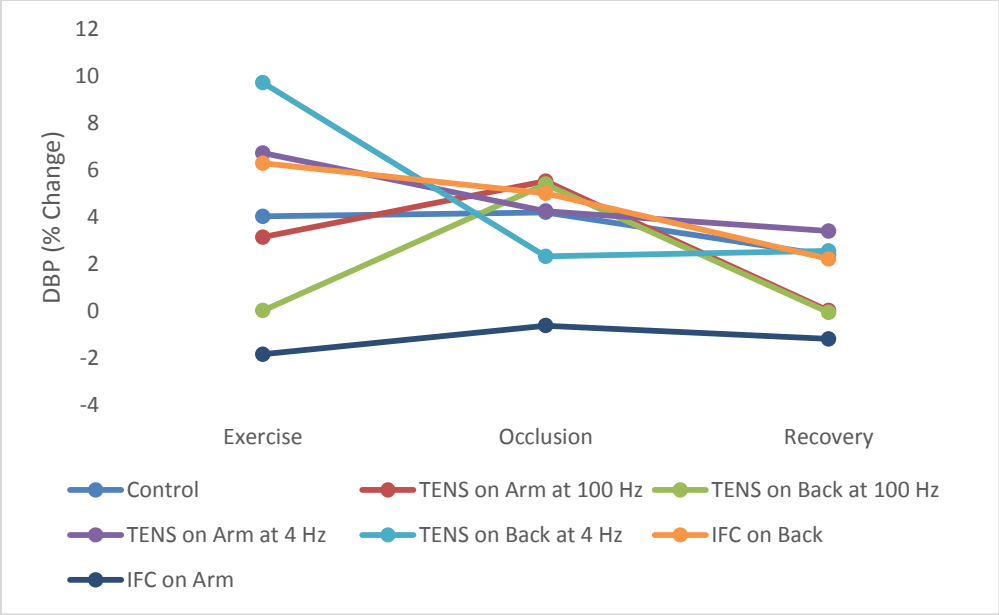

Figure 18. Percent change in DBP based on the treatment method. Differing patterns based on the treatment parameters were observed. IFC on the arm had a much lower percent change in DBP compared to the other treatments. TENS on the back at 4 Hz, displayed an unusual pattern, similar to the pattern it presented in SBP.

MAP was calculated using SBP and DBP to give an estimation of the pressure experienced in the vessels. There were no differences between the treatments' effect on MAP, but there was a trend showing an increase during occlusion, which was expected, **Figure 19**. It was expected that MAP would be decrease with the application of neurostimulation compared to the control.

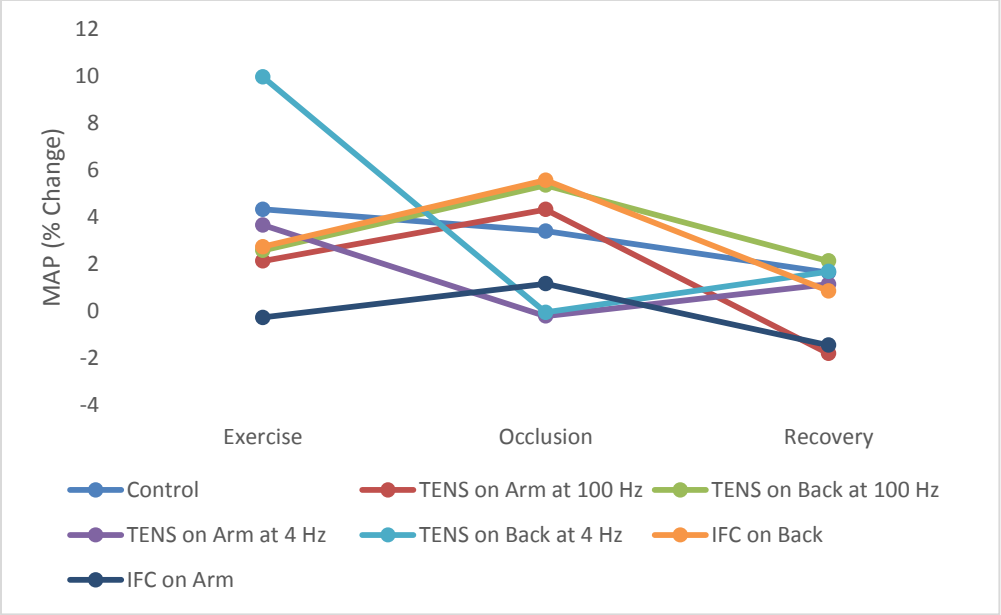

Figure 19. Changes in MAP based on the treatment method. IFC on the back as well as TENS at 100 Hz on the back increased MAP, while the greatest decrease in MAP was witnessed in IFC on the arm. *Note: approximated MAP

Heart rate, which was measured at the same time as blood pressure, showed differing patterns as well based on the treatment, **Figure 20**. There were no differences between any of the treatments, and there was no identifiable trend. We were not expecting to find any differences from control.

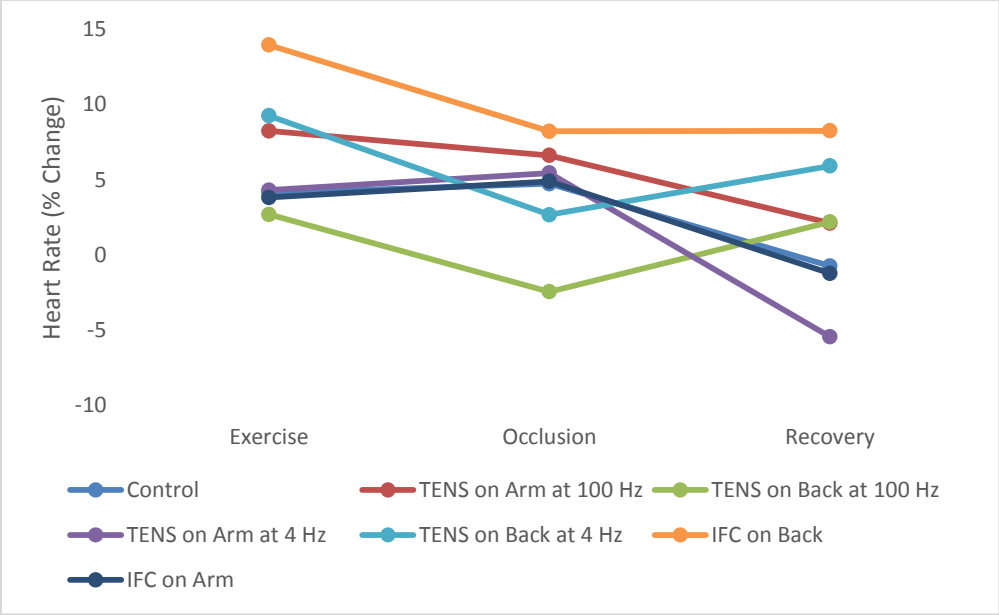

Figure 20. Percent change in heart rate due to treatment method. IFC on the arm had very similar percent changes to control, while the majority of the treatments showed a different pattern from the control. Of all the treatment methods, IFC on the back had the highest increase compared to control.

CVR in the palm was compared between all the treatment methods, **Figure 21**. CVR was expected to decrease, and it tended to increase during occlusion and decrease again during recovery. All treatments the followed a characteristic trend; however, IFC on the arm actually performed worse than the control. This means that there was more resistance in the vessels, making it harder for blood to flow to the desired region.

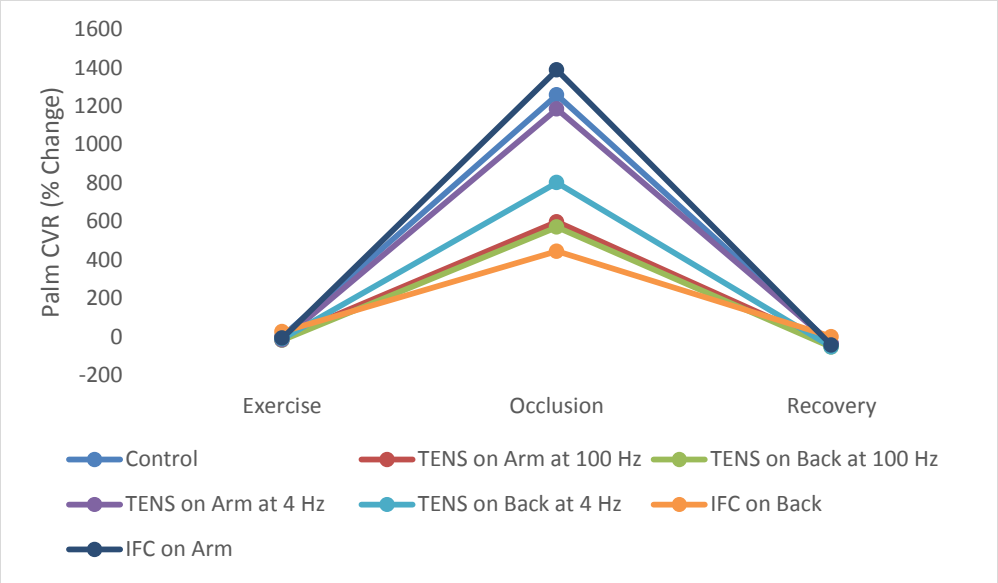

Figure 21. Changes to CVR in the palm region due to treatment method. IFC on the back had the greatest decrease in CVR, indicating it allowed the most blood flow compared to all other treatments. IFC on the arm had the greatest increase over control, indicating more resistance to flow.

CVR in the brachial region yielded mixed results due to the variability of the perfusion readings, **Figure 22**. There were no significant differences, but there was a trend for decreased CVR during occlusion which increased again during recovery. CVR was not expected to differ from the control.

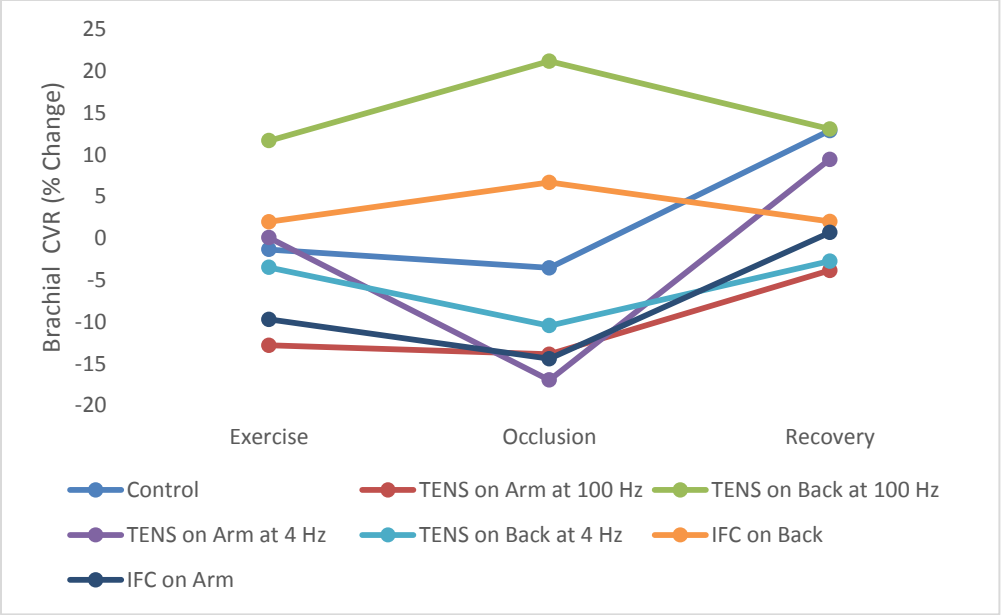

Figure 22. Changes to CVR in the brachial region due to treatment conditions. It was observed that brachial flow increased the most on average for TENS on the back at 100 Hz. IFC on the back also had higher than control results which then became lower during recovery. The other treatments caused lower percent changes in brachial blood flow compared to control, the lowest of which on average was TENS on the arm at 100 Hz.

Ischemic pain, which was measured using the NRS on a scale from 0-10, showed no differences in any treatment, but there was a trend indicating increased ischemic pain during exercise which was further amplified during occlusion, and decreased again during recovery as blood flow normalized, **Figure 23**. A pain rating of 0 indicated no pain, while 10 indicated the worst pain possible.

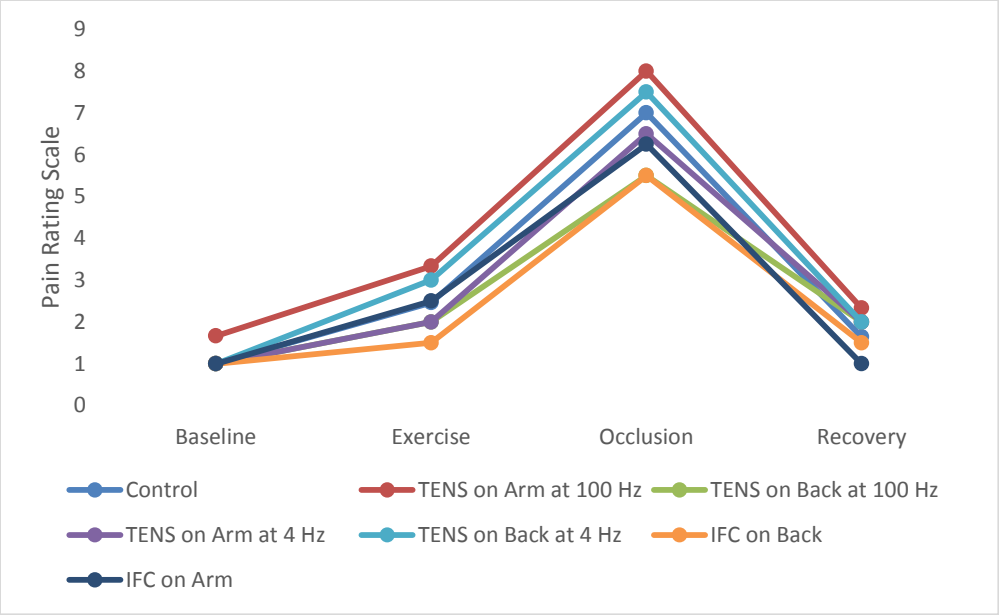

Figure 23. Change in ischemic pain levels correspond to treatment method. The lowest ratings were observed during IFC on the back. Both TENS on the back at 4 Hz and TENS on the arm at 100 Hz had higher pain ratings than the control.

Reperfusion time, or the time to recovery, was obtained by measuring the amount of time necessary for the blood perfusion to stabilize back to the baseline average during the recovery phase. There was a significant decrease in IFC on the back compared to all other treatment methods, **Figure 24** ($p<0.05$).

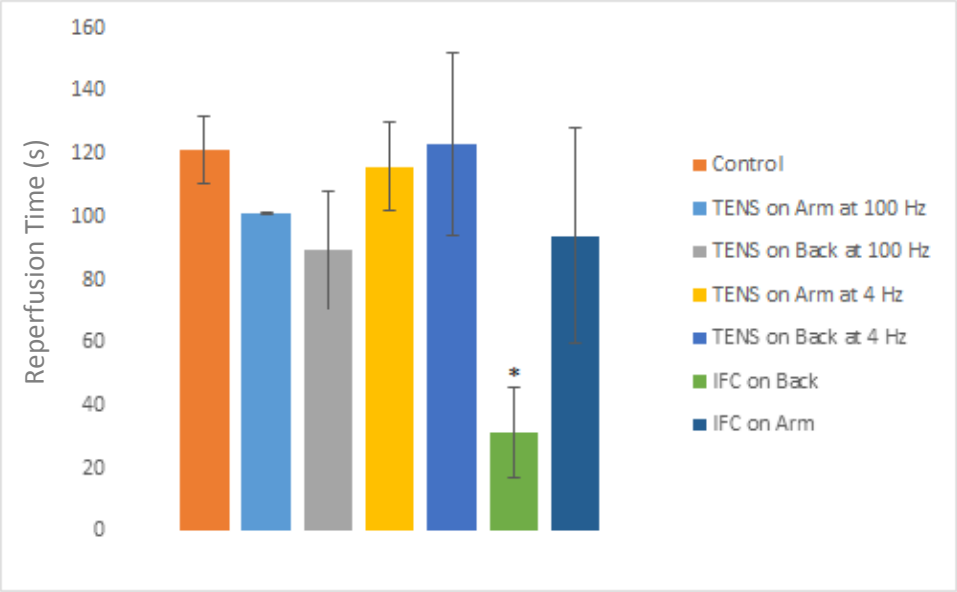

Figure 24. Average time to full recovery. IFC on the back had a statistically significantly reperfusion time. TENS on the back at 4 Hz had a slightly longer reperfusion time than control. * p-value < 0.05 compared to control.

CHAPTER 4: DISCUSSION

PAD is a painful disease caused by limited blood flow to the extremities. Many treatments used for this disease have side effects, but neurostimulation has been investigated for its ability to modulate pain and improve blood flow, especially in individuals with ischemic pain. The purpose of this study was to determine the ideal treatment for a future pilot study to increase perfusion and reduce ischemic pain. Once the ideal parameters have been determined, a full pilot study will be conducted to investigate the effect of the neurostimulation. To determine the best treatment, we measured perfusion in the palm, SBP, MAP, CVR in the palm region, ischemic pain, and reperfusion time based on the neurostimulation's inhibitory effect on the metaboreflex. The inhibition of the metaboreflex decreases sympathetic output. Although there were no significant changes from control in any of the treatments, almost all treatment groups demonstrated a trend towards increased perfusion. This suggests that TENS and IFC have the potential to reverse ischemia by increasing perfusion. MAP also had varying data because it was calculated from a static measurement, but IFC on the arm presented the greatest decreasing trend. All treatment groups expressed a decrease in CVR excluding TENS on the arm at 100 Hz; however, IFC on the back showed the greatest decrease. In addition, IFC on the back had the lowest reported ischemic pain and had a significant reduction in reperfusion time ($p<0.05$). From this data it is not possible to make a clear decision as to which treatment is the best for pilot work. IFC presented some positive pre-pilot results, though more pre-pilot work will need to be performed. In order to determine the ideal treatment method, all treatment modalities which performed better than control should be analyzed with a larger sample size.

Previous work using TENS and IFC to modulate pain and increase perfusion in individuals with ischemia has found some significant results, **Table III**. Several studies have concluded that low

frequency TENS significantly increases perfusion when applied to the arm compared to high frequency TENS and control [20, 24]. Based on the research performed in this current study, low frequency TENS applied to both the back and forearm had a trend towards increased palm perfusion compared to the control group. In addition, the increasing trend in blood flow for TENS at 100 Hz high frequency TENS was statistically similar to low frequency. An article expanded on the parameters tested, including MAP and CVR, while studying high intensity TENS. They found that there was increased blood flow when high frequency TENS was applied compared to the control as well as lower CVR, and a similar trend was found in this study [22]. IFCs effect on blood flow and ischemic pain has also been previously investigated [16, 21]. In this research, high frequency IFC produced the greatest increase and blood flow [21]. Although we were not comparing different IFC frequencies in this current study, we were able to see that IFC had lower MAPs and CVRs than control. These parameters can be correlated to blood flow. One article compared the use of high frequency TENS versus high frequency IFC, and found that both TENS and IFC caused significantly less ischemic pain during induced ischemia in healthy patients [16]. Interestingly, IFC and TENS at 100 Hz applied to the back tended to reduce ischemic pain while TENS at 100 Hz applied to the forearm tended to increase ischemic pain. One explanation for this is that the blood pressure cuff used to induce ischemia overlapped with the electrodes. TENS at 100 Hz creates a strong tingling feeling when applied to the forearm, and when the cuff was inflated it compressed the electrodes, which could have caused increased nocioception.

Table III – A Summary of Previous Study Results

Article	Measurement Information	Study Parameters	Measurements	Number of Subjects	Results
Cramp et al. 2002 [24]	Brachial and finger, healthy individuals	TENS 4 Hz	Blood flow	40	Significant increase from control
Cramp et al. 2000 [20]	Forearm, healthy subjects	TENS 4 Hz, 110 Hz	Blood flow	30	4 Hz showed significant increase
Vieira et al 2011 [22]	Calf, healthy subjects	TENS 80 Hz	Blood flow, MAP, CVR	22	TENS – higher blood flow, lower CVR, higher MAP during exercise
Noble et al. 2000 [21]	Quadriceps femoris, healthy subjects	IFC 80-100 Hz, 10-20 Hz	Blood flow	50	Higher frequency produced significant change
Johnson et al. 2003 [16]	Forearm, healthy subjects	IFC 100 Hz, TENS 100 Hz	Pain intensity	30	TENS, IFC had less pain

There were some limitations and possible causes for error during this research, including environmental factors such as cold temperature causing vasoconstriction and warm causing vasodilation. The effect of temperature was not analyzed in this data, and if body temperature changed during the testing, it could have inappropriately biased the results. In future testing, skin temperature will be measured at the beginning of each testing phase to ensure the effect of skin temperature is accounted for. Another problem was biofeedback; knowing how each of our hemodynamic parameters was supposed to change, could have inadvertently altered the results. Biofeedback can be reduced by inhibiting the individual's visual access to the testing results. A major limitation in this study was the small sample size (n=2). With such a small sample size, we did not have sufficient power to detect changes in our measured parameters. Similar studies tested between 22 and 50 individuals, but we were limited to testing on ourselves for pre-pilot

purposes. Ensuring individual comfort was also a challenge, particularly because any slight movement altered the readings on the laser Doppler system due to its sensitivity. Any motion artifacts seen in the raw data were removed from the trace to prevent false information. Discomfort could lead to artificially high hemodynamic parameters such as blood pressure and breathing. Through this study, it was determined that heavy breathing, over 50 mV strain on the ventilator belt, or irregular breathing patterns affected perfusion readings. Measures were taken to improve comfort such as a pillow, but sitting for long periods of time without moving can be difficult. Therefore, in future work a recumbent setup should be tested to see if it will reduce movement as well as how it affects blood flow. In addition to comfort, there were many factors that could affect the individual's results including lack of sleep, prior exercise, stress, and the consumption of stimulants such as caffeine and nicotine. These factors increase sympathetic output and could decrease the effectiveness of the treatment. The lack of continuous blood pressure measurement led to varied blood pressure results, making it harder to make conclusions about these treatments. Perhaps a continuous-measurement-system could be acquired for future research.

Other future work will include an investigation into the duration of the residual effects of the neurostimulation and investigating the systemic effects. Once additional pre-pilot work has been conducted, a full pilot study will be performed using the testing groups in Table I. Healthy students will be recruited and additional effects will be investigated such as skin temperature and sex.

CONCLUSION

TENS and IFC tend to have a favorable effect on hemodynamics and ischemic pain. IFC appeared to be the most effective neurostimulation modality, but it was not possible to make a conclusion as to which treatment method was able to produce the best results due to the need for additional replicates. Additional pre-pilot work will be continued to investigate this.

Once the pre-pilot work is completed, a pilot study followed by a full study will be conducted. The pilot study will use healthy college-aged students to investigate the effects of the selected neurostimulation modality on perfusion and ischemic pain, utilizing the 4 testing groups indicated in **Table I**. The pilot study will be the basis for a full research study investigating the effectiveness of the neurostimulation on individuals with PAD and ischemic pain. It is the hope that this will become a treatment used to alleviate the symptoms of PAD and improve quality of life.

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APPENDICES

Appendix A – Data Collection Systems

First the Powerlab system was configured and a custom settings file was created using four channels for: 1. Respiration Belt (Pneumotrace II Model #MLT1132/D), 2. Hand Dynamometer, 3. Distal Blood Perfusion and 4. Proximal Blood Perfusion. Blood perfusion was measured using the VMS-LDF2 Laser Doppler System and VP1T/7 skin probes (Moor Instruments). The readout of the Moor system was inspected to ensure perfusion measurements were being taken. Next, the volunteer was prepared for testing.

Appendix B – Average Values of All Parameters

	Exercise	Occlusion	Recovery
Control (n = 20)			
CBF 1	20.61408	-90.1396	70.7512
CBF 2	6.181795	10.56105	-7.52406
SBP	4.922132	2.513589	1.065731
DBP	4.045545	4.212532	2.391328
HR	4.116884	4.732632	-0.72447
MAP	4.343151	3.420077	1.658637
CVR 1	1.979512	1258.739	-36.7768
CVR 2	-1.34234	-3.54692	12.89023
*TENS Application on the Back at High Frequency (n = 6)			
CBF 1	25.43661	-81.5803	185.0897
CBF 2	-5.5738	-12.4239	-8.40812
SBP	6.04962	5.324572	5.120653
DBP	0.036075	5.411255	-0.03608
HR	2.698413	-2.43386	2.195767
MAP	2.595939	5.37299	2.147184
CVR 1	-18.2172	570.4271	-54.2402
CVR 2	11.68384	21.19491	13.07613
*TENS Application on the Forearm at High Frequency (n = 2)			
CBF 1	16.45631	-80.0942	114.3978
CBF 2	17.8305	24.17139	2.384457
SBP	0.81187	2.874207	-4.04069
DBP	3.159981	5.538713	0.034981
HR	8.249337	6.618037	2.122016
MAP	2.145089	4.337402	-1.7909
CVR 1	-5.08757	597.8964	-45.3314
CVR 2	-12.8027	-13.8696	-3.84971
*TENS Application on the Back at Low Frequency (n = 2)			
CBF 1	84.16821	-77.4402	259.6295
CBF 2	14.21466	14.37029	4.612285
SBP	10.29571	-3.0833	0.531915
DBP	9.732163	2.34357	2.574463
HR	9.254386	2.675439	5.921053
MAP	9.975071	-0.03917	1.698718

CVR 1	-10.7307	802.1998	-52.9969
CVR 2	-3.47213	-10.4482	-2.73357
TENS Application on the Forearm at Low Frequency (n = 2)			
CBF 1	44.57743	-82.7889	72.09013
CBF 2	5.046217	19.99818	-6.70987
SBP	0.423812	-5.45535	-1.87759
DBP	6.737288	4.265537	3.418079
HR	4.312039	5.454545	-5.42998
MAP	3.67893	-0.19509	1.161278
CVR 1	-16.3281	1184.719	-34.5453
CVR 2	0.07898	-16.9482	9.427779
*IFC Application on the Back (n = 2)			
CBF 1	-15.2391	-80.6055	3.330334
CBF 2	1.131429	-1.04201	-0.52675
SBP	-1.40911	6.140351	-0.90519
DBP	6.299841	5.023923	2.232855
HR	13.95161	8.225806	8.252688
MAP	2.753674	5.581614	0.869684
CVR 1	25.3756	445.1115	0.364934
CVR 2	1.982277	6.688098	2.013535
*IFC Application on the Forearm (n = 4)			
CBF 1	68.73909	-89.7606	117.8071
CBF 2	20.54841	37.41265	5.370384
SBP	3.632479	6.452991	3.076923
DBP	-4.3794	1.947623	-2.26673
HR	2.255727	8.625526	-1.6129
MAP	-0.92894	4.044313	0
CVR 1	-10.8726	1518.964	-43.7508
CVR 2	-16.7931	-20.5259	-2.40704