

EXAMINATION OF BLOOD FLOW USING VASOCONSTRICTOR AND
VASODILATOR STIMULI: STABILITY AND REPRODUCIBILITY

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
ABSTRACT.....	iv
CHAPTER	
1. INTRODUCTION.....	1
1.1 Study Purpose.....	2
2. METHODS.....	4
2.1 Participants.....	4
2.2 Experimental Design.....	4
2.3 Procedures.....	4
2.4 Experimental Measurements.....	9
2.5 Data Analysis.....	9
2.6 Statistical Analysis.....	11
3. RESULTS.....	12
3.1 Participant Characteristics.....	12
3.2 Vascular Response to Different Stimuli.....	13
3.3 Vascular Function Measures: Stability and Reliability.....	13
3.4 Correlations Between Vascular Measures.....	18
4. DISCUSSION.....	21
4.1 How Our Data Compare to Others.....	21
4.2 Physiological Pattern of Vascular Measures.....	23
4.3 Correlations Between Blood Flow Measures.....	28
4.4 Clinical Relevance.....	29
4.5 Limitations.....	30
4.6 Conclusion.....	30
REFERENCES.....	32
VITA.....	37

ABSTRACT

Blood flow distribution relies on the vasculature's ability to vasodilate and vasoconstrict throughout the body. Most previous research has focused on only one of these abilities, either vasodilation or vasoconstriction. For example, Thijssen et al. (2005) focused their research on vasodilation by studying reactive hyperemia, while Kinuyoshi et al. (2003) studied the possible vasoconstriction effect with increasing muscle sympathetic nerve activity. The purpose of this study was to assess vascular function using a variety of stimuli to potentially learn more about overall vascular health, while determining the stability and reliability of blood flow measurements using strain gauge plethysmography. Measures of vascular function were examined in 12 individuals [age=21±1 yrs]. Right lower leg resting arterial inflow, post occlusion reactive hyperemia, dynamic exercise blood flow, and blood flow following a cold stimulus were assessed on two separate occasions. The average resting arterial inflow was 2.27 ± 1.06 ml/100ml/min, reactive hyperemic blood flow was 19.42 ± 6.37 ml/100ml/min, exercise blood flow was 27.37 ± 14.95 ml/100ml/min, and blood flow following a cold stimulus was 1.53 ± 0.89 ml/100ml/min. A rather unique finding was the associations between the stimuli blood flow responses, by which those with the greatest reactive hyperemia blood flow responses also exhibited the greatest exercise blood flows and greatest drop in blood flow following the cold stimulus. In conclusion, the pattern of the blood flow responses and the correlations among the measurements, in addition to being stable and reliable, provide us with a greater understanding of the blood flow distribution properties of the vasculature.

CHAPTER 1. INTRODUCTION

Recent evidence points toward vascular dysfunction as an early identification of individuals at risk for cardiovascular disease (Katz et al., 2005; Ishibashi et al., 2006). Advancing non-invasive technology (e.g. Venous Occlusion Strain-Gauge Plethysmography and High-resolution, Ultrasonography) has focused on peripheral vascular function as a possible window through which vascular health may be identified. Particular protocols have been developed to stimulate a vascular response [occlusion (Alomari et al., 2003), cold (Dishman et al., 2003), mental stress (Sarabi & Lind, 2001), exercise (Tschakovsky et al., 2005), drugs (Benjamin et al., 1994), and heat (Fiscus et al., 2005)] which is then compared amongst individuals to possibly determine whose vascular function is compromised and what factors may be contributing to this compromise. The vascular response to the above-mentioned stimuli is often defined in terms of arterial inflow, venous capacitance, venous outflow, and arterial and/or venous resistance. These measures are generally obtained in the upper and/or lower limbs and are thought to help predict risk of mortality (Katz et al., 2005).

The majority of studies that have examined the vascular responses to different stimuli generally report significant differences in responses between those who are fit and unfit (Dishman et al., 2003; Alomari et al., 2003), with and without diseases such as hypertension (Olsen et al., 2004), coronary artery disease (Heitzer et al., 2003), diabetes (Rodriguez et al., 2003), and other pathological conditions (Katz et al., 1996; Zelis et al., 1974; Arnold et al., 1990), and young and old (Dobrosielski et al., 2006; Jasperse et al., 1994; Proctor et al., 1998). Moreover, growing evidence indicates that the outcome measures under investigation are modifiable through a variety of interventions including

exercise training (Alomari et al., 2003; Allen et al., 2003). Alomari et al. (2003) concluded that improvement in fitness level improves venous health, thereby improving the cardiovascular system. They stated that muscular conditioning may be important for venous health because of the association found between handgrip strength and venous system hemodynamics. Allen et al. (2003) saw improvements in brachial artery flow-mediated dilation after only four days of short-term handgrip exercise training.

A concern with the existing vascular studies, however, is that few have determined the stability and reliability of the measures. In addition, most studies determine vascular “health” using only one stimulus. Vascular health arguably depends on many different mechanisms. One impaired component does not imply a malfunctioning system, as other mechanisms may become more sensitive to compensate for the impaired component. On the other hand, it is critical to examine the role of vasoconstrictor and vasodilator properties during one visit in order to develop a better understanding of the integrated responses that are sure to determine the overall vascular health.

1.1 Study Purpose

The purpose of this study was to assess vascular function during a single visit using a variety of stimuli to potentially learn more about overall vascular health. Specifically, this study examined blood flow responses at rest, following occlusion, a single bout of exercise, and a cold stimulus, in this particular order. Of particular concern were the stability and reliability of the vascular flow responses as assessed with strain-gauge plethysmography. Whereas this study does not offer any hypotheses in terms of the stability or reproducibility of the responses, it is hypothesized that the flow responses will

be greater following occlusion and exercise when compared to rest. In addition, it is hypothesized that blood flow following the cold stimulus will be lower than those observed at rest.

CHAPTER 2. METHODS

2.1 Participants

Participants for this study were recruited from current students enrolled in laboratory classes in the Department of Kinesiology at Louisiana State University. Students were asked to complete a medical history questionnaire and physical activity questionnaire. The participants were approved according to the rules of the Institutional Review Board at Louisiana State University. Participants did not have any overt signs or symptoms of disease, and were non-smokers. Individuals who had undergone any recent medical/surgical procedures, or had evidence of disease, or were on medications known to influence the vasculature were excluded from participation. Each participant was required to complete an informed consent approved by the IRB at Louisiana State University for Kinesiology activity classes.

2.2 Experimental Design

The current study was a prospective and test-retest design. It included three visits, 1 through 3, of data collection. Visit 1 included a Symptom-Limited Graded Exercise Test (SLGXT) on a cycle ergometer and a one repetition maximum of the right calf muscle. Vascular function measurements were obtained during visit 2 and again during visit 3. The three separate visits used in this study are listed in Table 2.1 and further detailed in the next section.

2.3 Procedures

Visit 1. First, a medical health and physical activity questionnaire was administered once an informed consent had been signed. Next, the participant was asked to complete a maximal voluntary contraction of the right calf muscle. This included right

plantar flexion against a loaded pedal (Figure 2.1) until volitional fatigue was reached. Fatigue was defined as the inability to lift the load one time through the full range of motion. On the average, approximately 3 to 5 trials per person was attempted before failure was reached. Participants were given 30 to 60 seconds of rest between trials. Once this maximum was recorded, the participant was asked to complete a SLGXT on a cycle ergometer. The participant was required to have a meal before this visit due to the exercise protocol included. This test consisted of a resting baseline measurement of five minutes, followed by a cycle ergometer exercise protocol. The exercise protocol required the participant to maintain 50 revolutions per minute beginning at 0 kilopounds, while the tester increased the workload by twenty-five Watts every two minutes until the participant requested to terminate the test or could not maintain 50 revolutions per minute. Once the exercise protocol was complete, the participant recovered for five minutes, while at rest.

Visit 2. This visit consisted of taking blood flow measurements of the right leg during varying stimuli using strain-gauge plethysmography, with each participant being asked to refrain from exercise and alcohol for twenty-four hours and from food, medication, and caffeine consumption for ten hours. The participant was also asked to bring a pair of shorts to wear during the testing. The vascular stimuli consisted of an upper leg occlusion condition, a dynamic exercise condition of right calf flexion, and a condition where the left foot was submerged in ice water. Prior to each of the vascular stimuli, resting blood flow measures were obtained. Throughout the vascular assessment, the participant remained in a supine position. Prior to any measure, the participant was allowed to relax for 15 minutes. Throughout the experiment, blood pressure and heart

rate were monitored. In between each vascular stimulus the participant was given an additional rest period of 5 minutes to allow for baseline values to be restored.

All arterial inflow measurements were obtained using previously published and established protocol (Bleeker et al., 2003; Groothuis et al., 2003). Briefly, at each point of measurement, the investigators recorded three ten second measurements, with ten seconds of rest between measurements. The resting measurement included arterial inflow following one minute of ankle occlusion. This measurement was taken before each condition to assess baseline blood flow. The occlusion condition included five minutes of thigh occlusion using a suprasystolic pressure of 80mmhg above systolic pressure. After the period of occlusion, reactive hyperemia was taken, as described by Thijssen et al. (2005). With regards to the exercise condition, a dynamic exercise stimulus was used. We were particularly interested in the vascular response during exercise, and according to Laughlin (1999), blood flow increase during dynamic exercise is due to decreased vascular resistance in the muscle. However, according to the same study, blood flow during isometric exercise, in addition to increasing central nervous system, only increases up to 30% maximal voluntary contraction, which suggests that properties other than the vascular responses help control blood flow. In addition, the muscle pump mechanism, unknown vasodilators, and, to a lesser extent, functional sympatholysis are believed to play large roles in blood flow increase within 30 seconds of dynamic exercise (Tschakovsky & Sheriff, 2004), which was an integrated response in the vasculature that we were interested in testing. Therefore, the dynamic exercise stimulus was adopted. Given the time frame needed to induce such responses following dynamic exercise, participants were instructed to perform right plantar flexion against a loaded pedal (60%

of their maximal voluntary contraction) for fifteen contractions using a one-to-one second cadence. The protocol used was a modification of a previously reported study protocol for upper limb exercise (Van Beekvelt et al., 2001). Upon the thirteenth contraction, the ankle cuff was inflated, while after completion of the fifteenth contraction, the rapid inflating thigh cuff was inflated to measure arterial inflow.

The foot submersion test included a one-minute submersion of the left foot in ice water, while the right foot was occluded from any blood flow. Then, after the one-minute foot submersion, the thigh cuff was inflated to measure arterial inflow. The entire protocol for Visit 2 may be seen in Table 2.2.

Visit 3. This visit used the same method as described in Visit 2 (Table 2.2), only this visit served as a retest one week after visit two.

Table 2.1 Participant Visit Description

Visit	Assessment
Visit 1: Familiarization	<ol style="list-style-type: none"> 1. Medical Health Questionnaire 2. Physical Activity Questionnaire 3. Maximal Voluntary Contraction 4. Symptom-Limited Graded Exercise Test <ol style="list-style-type: none"> a. Rest b. Graded response c. Recovery
Visit 2: Vascular Function Assessment	<ol style="list-style-type: none"> 1. Resting Arterial Inflow 2. Reactive Hyperemic Blood Flow 3. Dynamic Exercise Blood Flow 4. Cold Pressor Test Blood Flow
Visit 3: Vascular Function Assessment	<ol style="list-style-type: none"> 1. Resting Arterial Inflow 2. Reactive Hyperemic Blood Flow 3. Dynamic Exercise Blood Flow 4. Cold Pressor Test Blood Flow

Table 2.2 Visit 2 and 3 Protocol

Stimulus	Protocol
Rest	<ol style="list-style-type: none"> 1. Inflate ankle cuff to 240mmHg for 1 minute, and measure blood pressure. 2. Inflate rapid thigh cuff to 7mmHg below diastolic blood pressure while recording arterial inflow for 10 seconds. 3. Repeat this measurement twice after ten seconds of rest each. 5. Deflate all cuffs.
Upper Leg Occlusion	<ol style="list-style-type: none"> 1. Inflate thigh cuff to suprasystolic pressure for 5 minutes. 2. After 4th minute, inflate ankle cuff to 240mmHg, then measure blood pressure. 3. Clamp scissors on rapid cuff tube to maintain pressure, then set the thigh pressure to 7mmHg below diastolic. 4. After 5th minute of occlusion, release scissors from tube and measure arterial inflow for 5 seconds. 5. Deflate thigh cuff for 10 seconds, then measure arterial inflow for 10 seconds, and repeat once more. 6. Deflate all cuffs.
Dynamic Exercise (60% MVC)	<ol style="list-style-type: none"> 1. Dynamic plantar flex the foot for 15 contractions (1/1 second flex/rest cadence) 2. During 13th contraction, inflate ankle cuff to 240mmHg. 3. During 15th contraction, inflate rapid thigh cuff to 7mmHg below diastolic, 1% balance plethysmograph, and measure arterial inflow for 10 seconds. 4. Relax thigh cuff for 10 seconds and repeat arterial inflow measurement for 10 seconds. (Repeat for 3rd measurement) 5. Deflate all cuffs.
Cold Pressor Test	<ol style="list-style-type: none"> 1. Submerge left foot in ice water for 1 minute, while also inflating ankle cuff to 240mmHg. 2. Take BP & HR. 3. At the end of 1 minute, inflate rapid thigh cuff to 7mmHg below diastolic and measure arterial inflow for 10 seconds. (Repeat twice more with 10 seconds of rest between measurements) 4. Deflate all cuffs.

2.4 Experimental Measurements

The maximal voluntary contraction measurement was determined using the device shown in Figure 2.1, which allowed the participant to plantar flex the foot at a 30 degree angle. In addition, whole-body maximum and peak VO₂ gas analysis were examined on a cycle ergometer. Blood flow measurements began with measuring calf circumference to determine the strain-gauge size for the participant. Venous occlusion strain-gauge plethysmography was used to assess blood flow changes in the vasculature of the right leg during the different conditions (E-4 Hokanson, Hokanson, Bellevue, WA). Reproducibility of this measurement technique has been well-documented (Thijssen et al., 2005; Alomari et al., 2004; Tschakovsky et al., 1995; Groothuis et al., 2003). During all conditions, only the arterial flow was measured.

A rapid-inflating pressure cuff on the right thigh was used during strain-gauge plethysmography to occlude blood vessels in the leg (E-20 Hokanson Rapid Cuff Inflator). Meanwhile, a wrist pressure cuff was used around the right ankle to occlude blood flow to the foot. This method is used to ensure that the circulation being measured is strictly in the calf, as Lenders et al. (1991) presented with use in the forearm.

Blood pressure and heart rate was measured before and throughout each condition. The blood pressure measurements were used to determine the pressure needed by the rapid-inflating pressure cuffs to occlude the venous or arterial flow, depending on which test was being administered.

2.5 Data Analysis

The gas analyses were recorded as a maximum and a peak. Maximum VO₂ was calculated by averaging the last thirty seconds of analysis, while peak VO₂ was

calculated by averaging the three highest breath-by-breath measurements. Blood flow measurements were analyzed using a formula derived from the plethysmograph scale. Using .5 per cent range on the plethysmograph, the space between the bottom line and the top line represents 4centimeters. By measuring the distance from the baseline of the blood flow slope to the blood flow slope at a specific time on the plethysmograph, it is possible to determine the blood flow rate at this point in time by dividing the distance (in centimeters) by the time (in seconds), then multiplying this number by .5 (per cent range) and 60 (seconds in one minute). This formula may be seen below.

$$\text{Blood Flow} = d * 1/t * .5 * 60$$

The blood flow rates are measured in $\text{ml} * 100\text{ml tissue}^{-1} * \text{min}^{-1}$. In addition, reactive hyperemia and exercise blood flows were calculated by multiplying blood flow rates by 3. This calculation was found through conversion of fast plethysmograph speed into slow speed. Finally, once the three ten second measures for each stimulus (recall Table 2.2) were calculated for each participant, the mean of the measurements withineach stimulus was recorded as our results, giving us the average blood flow.

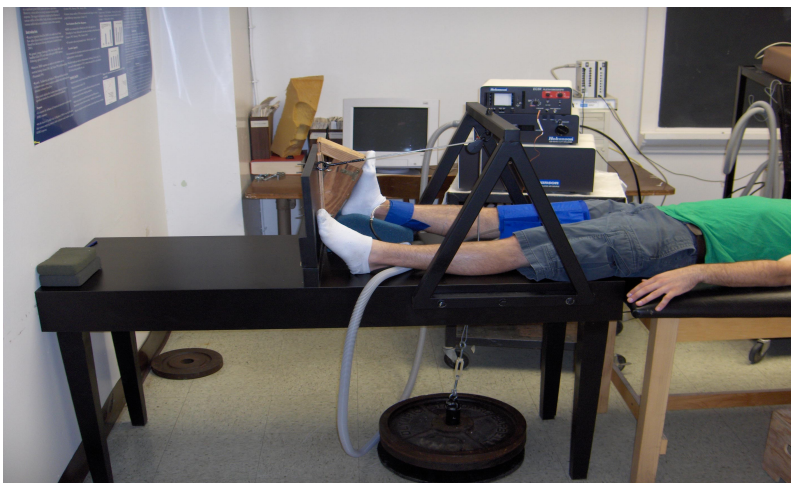


Figure 2.1 Device used for exercise

2.6 Statistical Analysis

Data is presented as means and standard deviations. The stability of the measurements was demonstrated by calculating the mean difference between visits for each stimulus. To examine the pattern of flow responses to the various stimuli, univariate analysis of variance was used, in which blood flow was the dependent variable and the stimuli was the independent variable. In addition, univariate analysis of variance was used to examine the between-visit differences of the stimuli responses, in which blood flow was the dependent variable and the stimuli by visit was the independent variable. To examine the reliability between individual measures, simple Pearson Product Moment Correlation was used, while an Interclass Correlation Coefficient was used to assess the reliability of our vascular assessments during one, 60 minute protocol. Finally, Pearson Product Moment Correlation was used to examine the relationships between the different stimuli.

CHAPTER 3. RESULTS

3.1 Participant Characteristics

Twelve adults (8 Men and 4 Women) between the ages of 20 and 24 years old participated in this study. Participant characteristics for age, height, weight, systolic blood pressure, diastolic blood pressure, resting heart rate, and strain gauge size, pre-cold pressor test heart rate (PreCPT HR), and cold pressor test heart rate (CPT HR) are expressed in Table 3.1. Table 3.2 contains fitness characteristics for maximum plantar flexion (1RM), whole-body peak and maximal oxygen consumption and maximum exercise heart rate (HR_{ex}) and peak exercise rating of perceived exertion. In addition, the self-reported physical activity questionnaires revealed participation in physical activity to be an average of 4 days per week.

Table 3.1 Participant Characteristics

	N	Minimum	Maximum	Mean	SD
Age (yrs)	12	20	24	21	1
Height (cm)	12	162	188	174	10
Weight (kg)	12	55	119	73.62	17.15
SBP rest (mmHg)	12	100	137	112	10
DBP rest (mmHg)	12	55	85	69	9
HR rest (bpm)	12	48	84	62	12
PreCPT HR (bpm)	24	47	86	62	11
CPT HR (bpm)	24	50	90	66	12

Table 3.2 Fitness Characteristics

	N	Minimum	Maximum	Mean	SD
1RM (kg)	12	52	86	61	12
VO₂peak (ml/kg/min)	12	30	48	39	6
VO₂max (ml/kg/min)	12	27	48	37	7
HR_{ex} (bpm)	12	180	200	188	6
RPE	12	17	20	19	1

3.2 Vascular Responses to Different Stimuli

Mean vascular function measurements are shown in Table 3.3. The mean calf arterial inflow at rest was 2.27 ± 1.06 ml/100ml/min and increased to 19.42 ± 6.37 ml/100ml/min post occlusion. The mean calf pre-exercise arterial inflow was 1.86 ± 1.06 ml/100ml/min and increased to 27.37 ± 14.95 ml/100ml/min post exercise. The mean calf pre-cold pressor test arterial inflow was 2.26 ± 1.23 ml/100ml/min and decreased to $1.53 \pm .89$ ml/100ml/min following the cold stimulus. The mean vascular responses for all the participants are illustrated in Figure 3.1. In addition, a univariate analysis of variance, using blood flow as the dependent variable and the stimuli as the independent variable, revealed significant differences, in one model, between the vascular stimuli and resting blood flows ($p=.0001$). These differences are indicated in Figure 3.1.

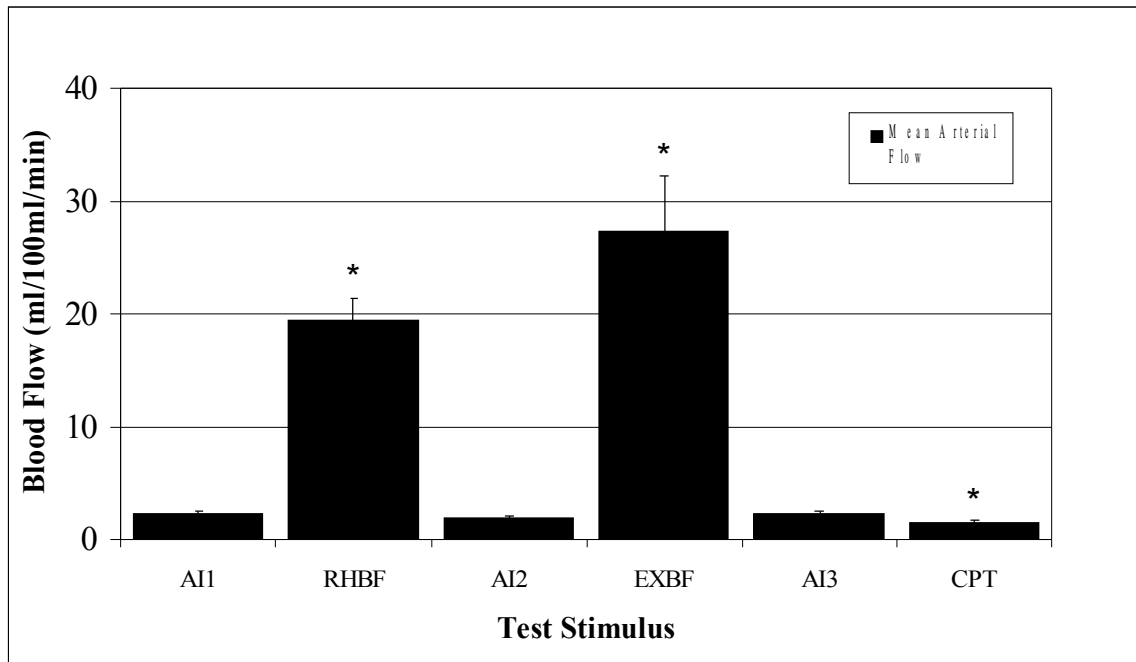
Table 3.3 Mean Vascular Function Measurements

Blood Flow (ml/100ml/min)	N	Minimum	Maximum	Mean	SD
Arterial Inflow 1	24	0.9	4.45	2.27	1.06
Reactive hyperemia	24	7.59	32.63	19.42	6.37
Arterial Inflow 2	24	0.53	4.5	1.86	1.06
Exercise Blood Flow	24	10.8	62.7	27.37	14.95
Arterial Inflow 3	24	0.3	4.7	2.26	1.23
Cold Pressor Test	24	0.52	4.05	1.53	0.89

3.3 Vascular Function Measures: Stability and Reliability

The stability of our mean vascular function measurements for visit 1 and 2 may be seen in Table 3.4 and Figures 3.2 and 3.3. The mean differences for reactive hyperemia, exercise blood flow, and blood flow following the cold stimulus were 2 ml/100ml/min, 1.97 ml/100ml/min, and 0.16 ml/100ml/min, respectively. Univariate analysis of variance, using blood flow as the dependent variable and stimuli by visit as the independent variable, revealed that there was, in one model, no between-visit differences

for the stimuli responses ($p=.989$). The Pearson Product Moment Correlations between the individual measurements of RHBF, EXBF, and CPT are presented in Table 3.5 and are illustrated in Figures 3.4, 3.5, and 3.6, with Figure 3.7 further demonstrating the stability of the CPT blood flow measures and changes from rest (CPTCH) during Visit 1 and Visit 2. The overall Interclass Correlation Coefficient for our vascular function assessments in one protocol (Figure 3.1) was 0.61 (95%CI = 0.307 – 0.81; $P=.001$).



*Significant difference vs. AI and Vascular Stimuli (RHBF, EXBF, and CPT) at ($\alpha=.05$)

Figure 3.1 Mean Vascular Responses

Table 3.4 Visit 1 & 2 Mean Vascular Function Measurements

Blood Flow (ml/100ml/min)	Visit 1		Visit 2	
	Mean	SD	Mean	SD
Arterial Inflow 1	2.34	1.06	2.21	1.1
Reactive Hyperemia	20.44	6.3	18.4	6.55
Arterial Inflow 2	1.99	1.21	1.73	0.92
Exercise Blood Flow	28.36	16.42	26.39	13.98
Arterial Inflow 3	2.38	1.41	2.15	1.07
Cold Pressor Test	1.61	0.96	1.45	0.85

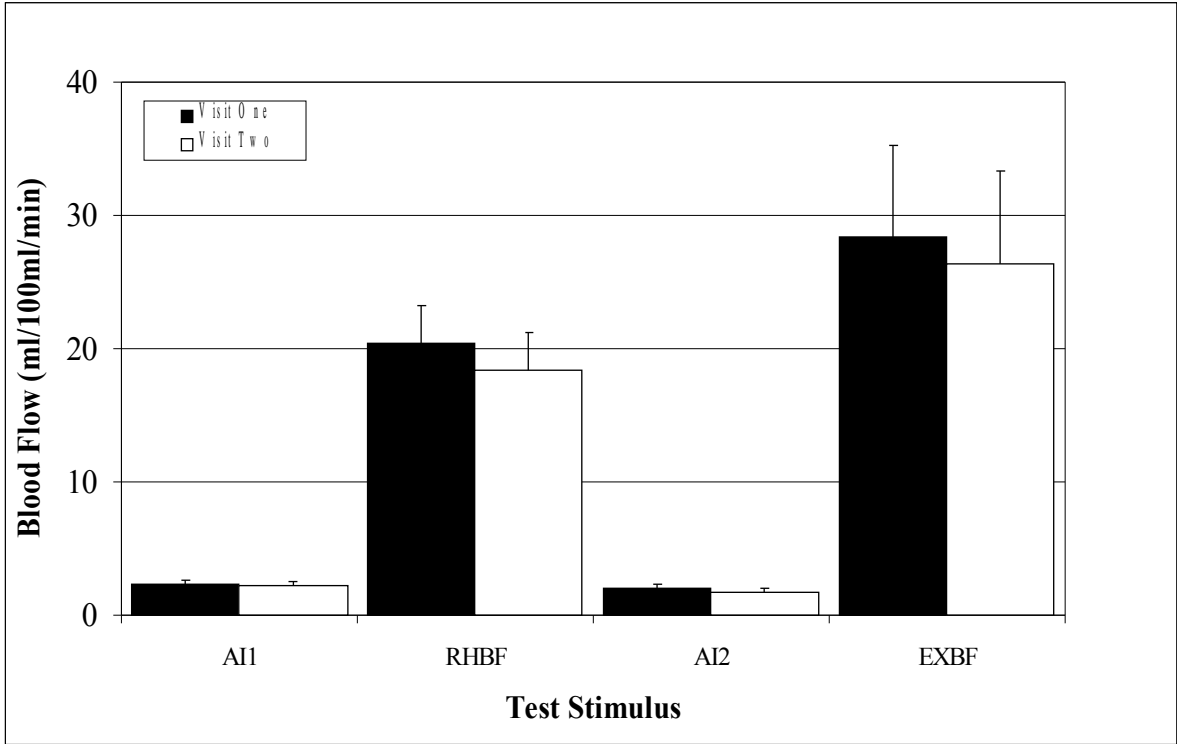


Figure 3.2 AI 1, RHBF, AI 2, and EXBF for Visit 1 versus Visit 2

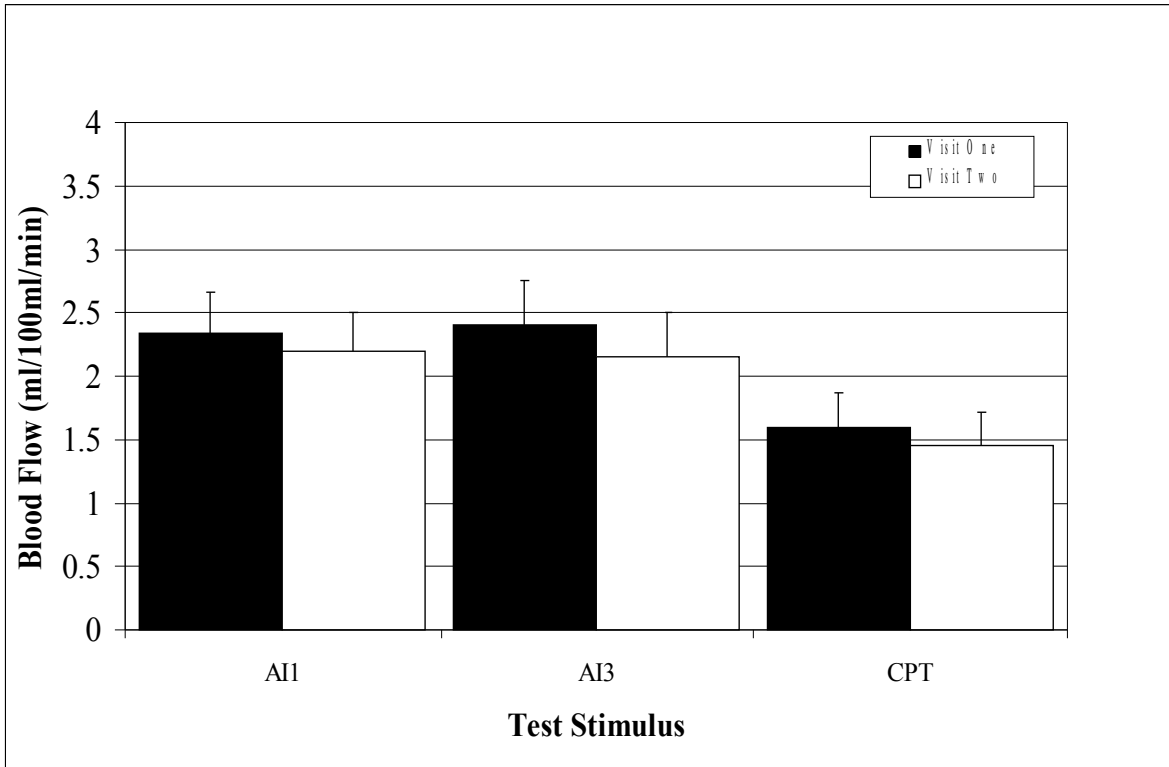


Figure 3.3 AI 1 & 3 and CPT blood flow for Visit 1 versus Visit 2

Table 3.5 Correlations Between Individual Measures of RHBF, EXBF, and CPT

	Measure 1	Measure 2	Measure 3
RHBF	0.68	0.56	NA
EXBF	0.6	0.75	0.71
CPT	0.59	0.65	NA

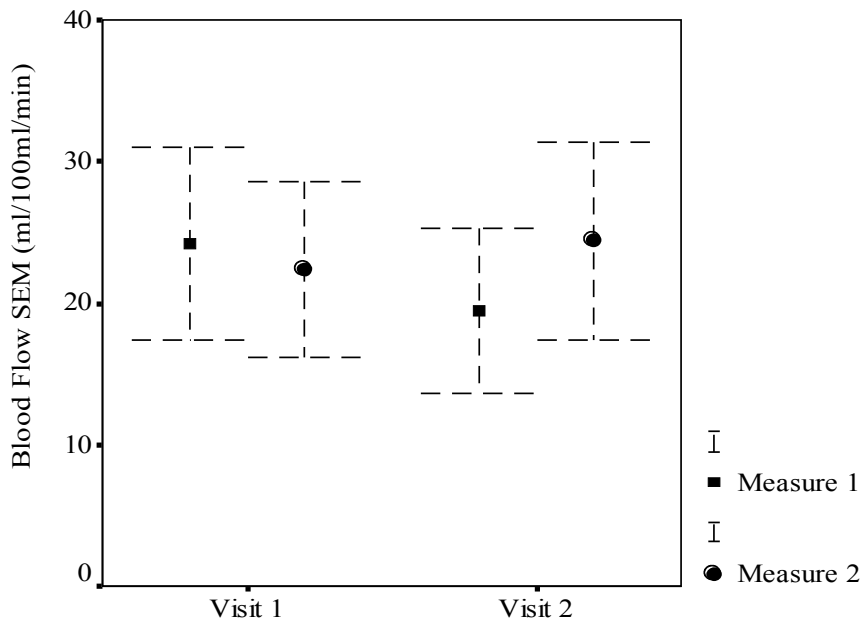


Figure 3.4 Individual measures of Reactive Hyperemia

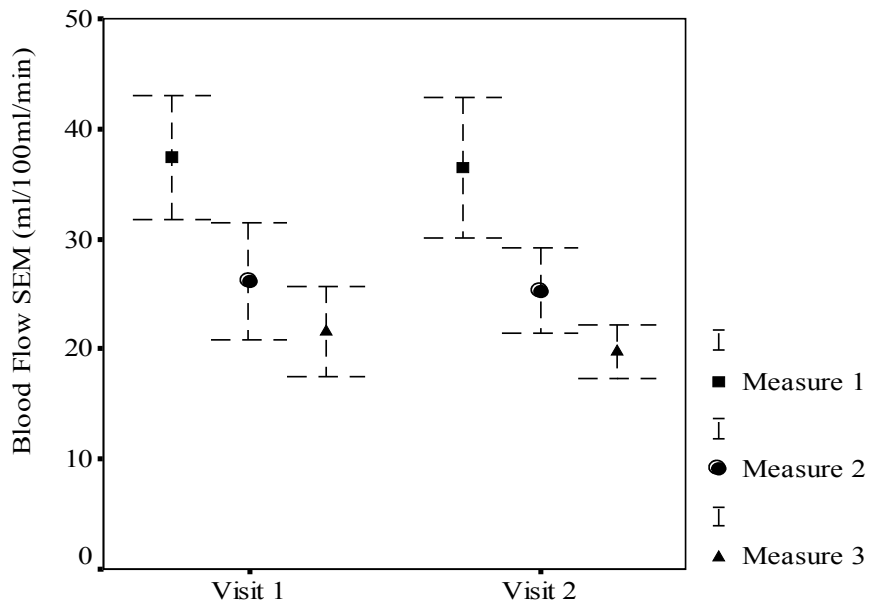


Figure 3.5 Individual Measures of Exercise Blood Flow

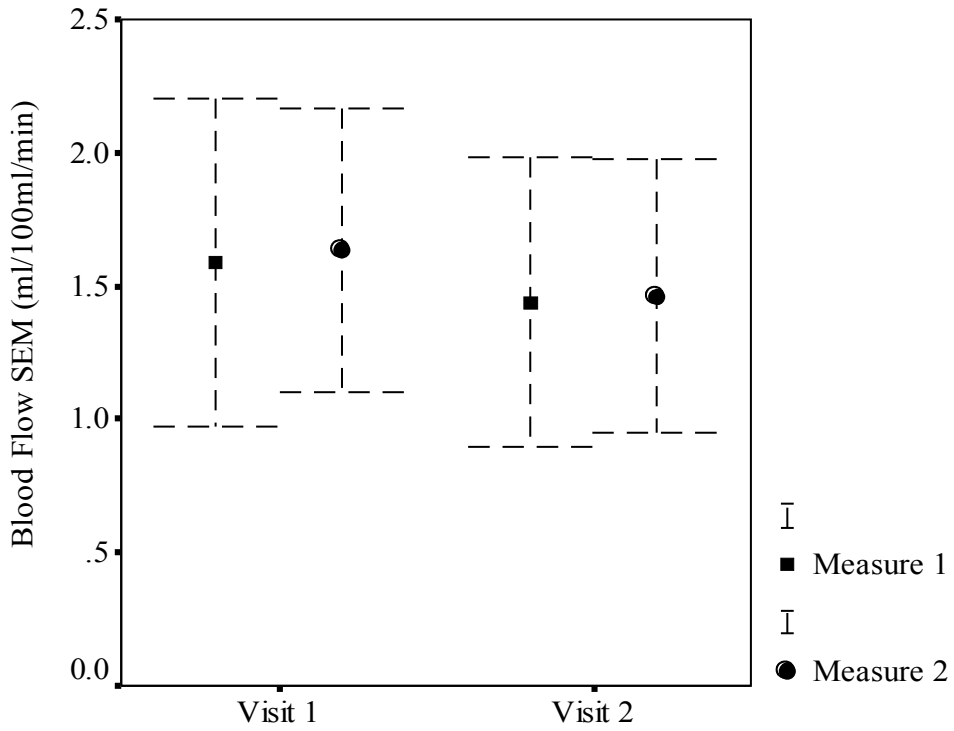


Figure 3.6 Individual Measures of CPT Blood Flow

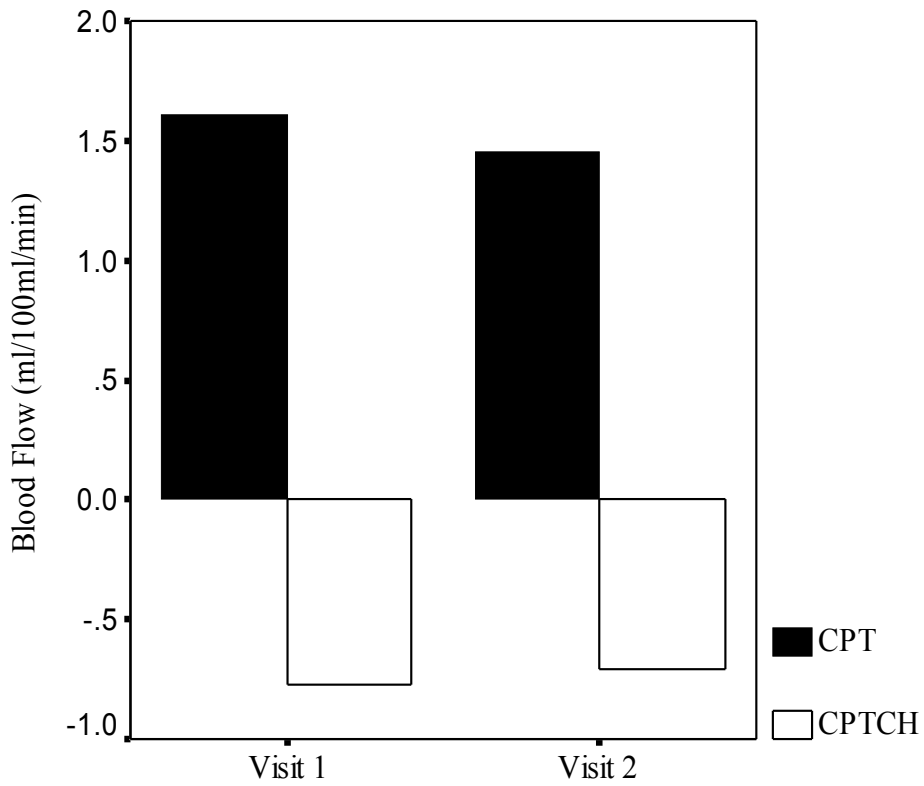


Figure 3.7 Stability of CPT/CPTCH Measures

3.4 Correlations Between Vascular Measures

Examination of the relationships between the vascular measures revealed distinct correlations among our vascular measures, which may be seen in Figure 3.8, 3.9, and 3.10. There was a significant correlation between reactive hyperemia and both exercise blood flow (EXBF) and blood flow change from rest to following the cold stimulus (CPTCH) ($\alpha=.05$). These correlations are illustrated in Figures 3.8 and 3.9, respectively. In addition, there was a correlation between EXBF and CPTCH ($\alpha=.06$), which is depicted in Figure 3.10.

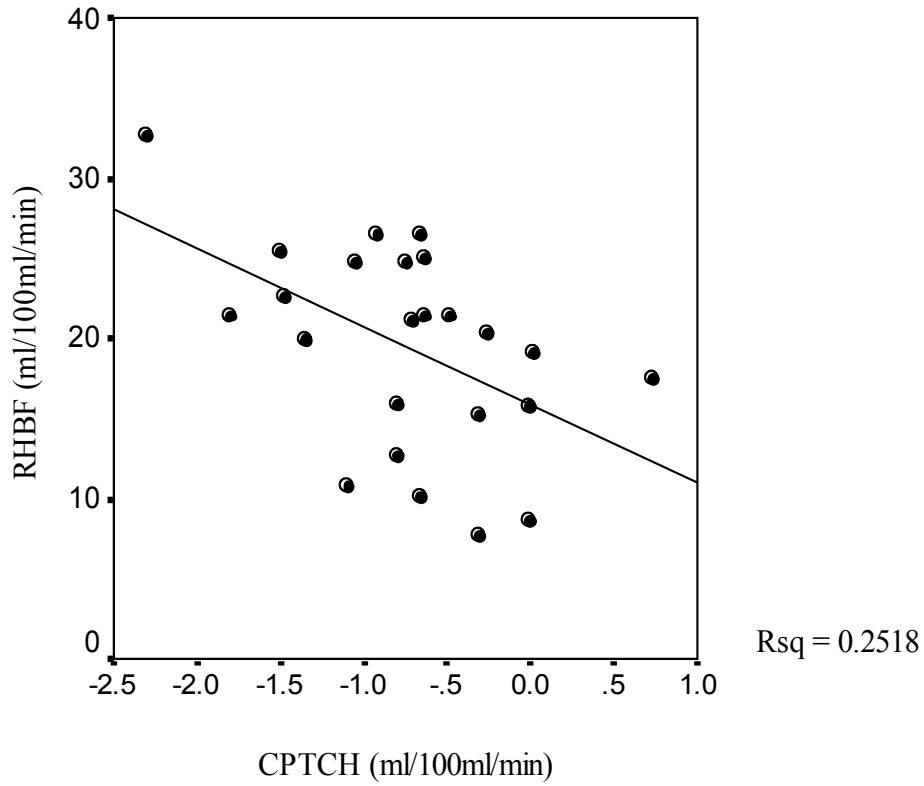


Figure 3.8 Correlation Between RHBF and CPTCH

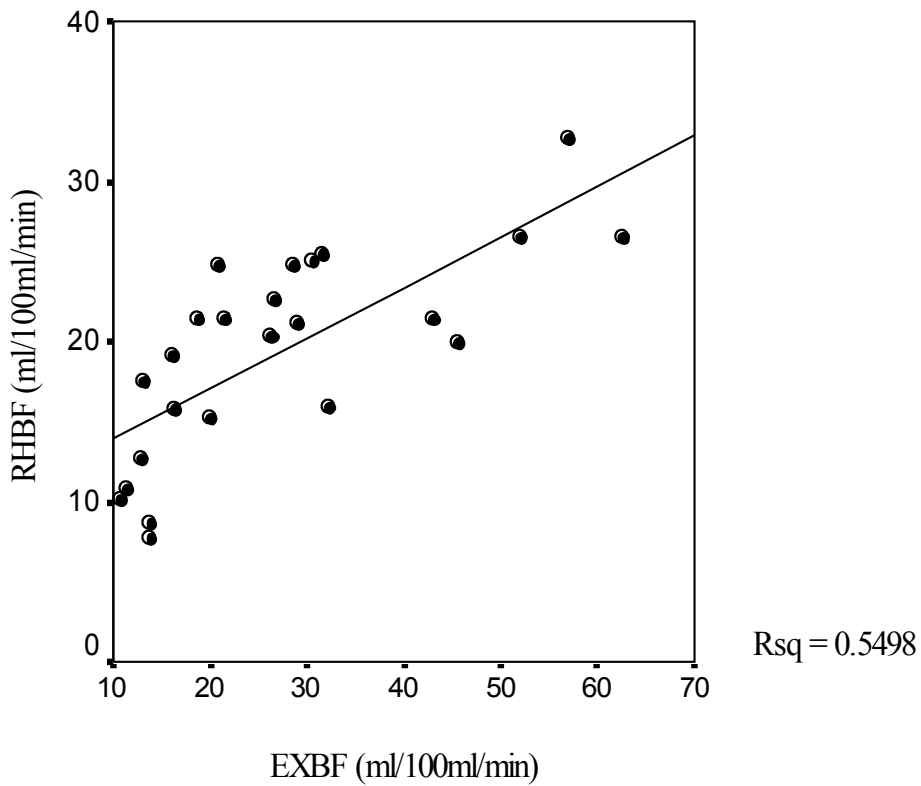


Figure 3.9 Correlation Between RHBF and EXBF

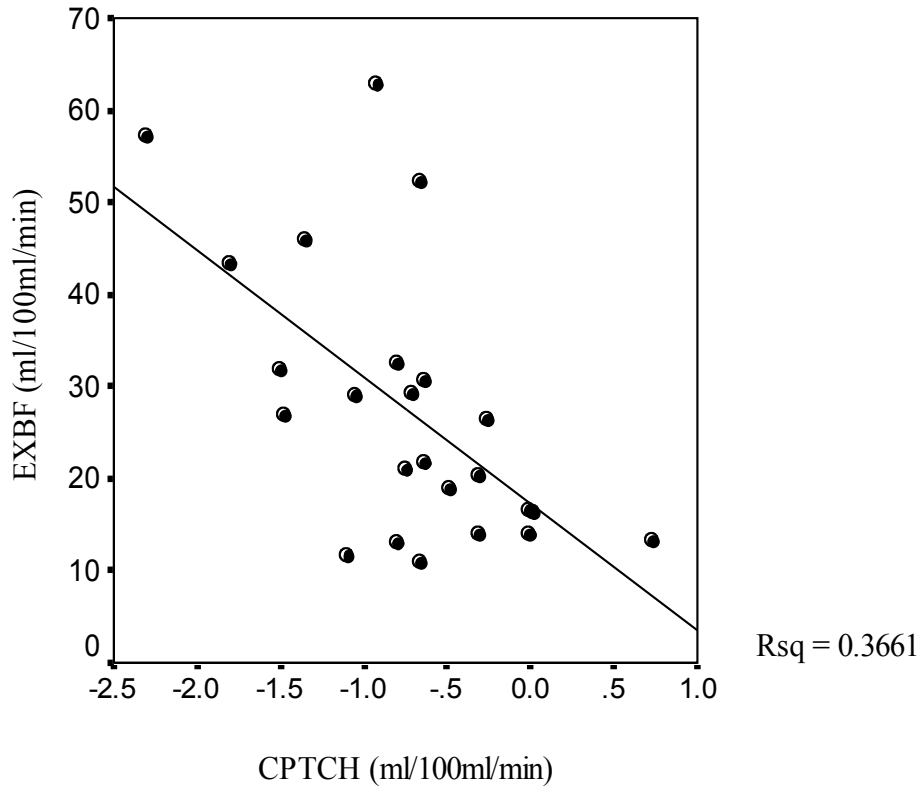


Figure 3.10 Correlation Between EXBF and CPTCH

CHAPTER 4. DISCUSSION

The purpose of this study was to assess vascular function using a variety of stimuli to potentially learn more about vascular health, while also determining the stability and reliability of the vascular flow responses as assessed with strain-gauge plethysmography. The unique findings of this study are the patterned response presented by the vasculature following the different stimuli and the correlations among our different vascular measures. The pattern we presented in this study demonstrates that the dynamic exercise stimulus resulted in the highest blood flow response, followed by reactive hyperemia, then resting blood flow, with the cold stimulus resulting in a blood flow response lower than that of rest. In addition to this pattern, we found correlations between increased reactive hyperemia and both increased dynamic exercise blood flow and decreased blood flow following the cold stimulus.

4.1 How Our Data Compare to Others

Vascular measurements in our study are consistent with previous findings. Most previous studies have found resting blood flow to be near 2.5 ml/100ml/min (Thijssen et al., 2005; Groothuis et al., 2003; Williams & Leggett, 1989). Thijssen et al. (2005) and Groothuis et al. (2003) found resting arterial inflow to be near 2.5 ml/100ml/min. Williams and Leggett (1989) compiled several studies between 1969 and 1982, all of which were between 2.0 and 2.7 ml/100ml/min. Polichnowski et al. (2005) found, however, a lower resting arterial inflow near 1.6 ml/100ml/min. The current study included three mean resting arterial inflow measurements of 2.27, 1.86, and 2.26 ml/100ml/min, all of which are between the previously reported measurements.

In regards to the reactive hyperemic blood flow responses, data from the present study are very similar to the upper limb responses seen in other studies from our laboratory (Alomari et al., 2003; Tisdell, 2004). Though the present results are lower than the reported value of 31 ± 5 ml/100ml/min by Thijssen et al. (2005), which used similar procedures, our reactive hyperemic data does follow a near ten-fold increase in blood flow from rest following occlusion, which is generally expected in healthy individuals who are moderately fit (Joyner et al., 2001).

The blood flow responses following exercise are difficult to compare to other studies due to differences in the stimuli used. In fact, there does not appear to be consistency in the exercise stimulus as it relates to intensity and duration. However, despite these inconsistencies between studies, the present average exercise arterial inflow of 27.37 ± 14.95 ml/100ml/min is very similar to that reported by Laaksonen et al., (2003), who reported average flows of 25 ± 3.9 ml/100ml/min. In comparison to Boushel et al. (2000), the findings in the present study are significantly lower. Boushel et al. (2000) reported average exercise blood flows of 44 ml/100ml/min following five minutes of dynamic plantar flexion using a workload of 9 Watts. However, the same study did find calf blood flow to be near 30 ml/100ml/min after five minutes of dynamic calf flexion using a workload of 5 Watts. Thus, it would appear that the exercise flow responses are very much dependent on exercise duration and intensity.

Finally, the average flow responses to the cold pressor test are very much within the range of previous findings in forearm blood flow. In the majority of studies that have examined healthy and younger individuals, the decrease in blood flow ranges from -0.3 to -0.8 ml/100ml/min (Dishman et al., 2003; Kinuyoshi et al., 2003). It is recognized that

the flow responses are dependent on a variety of factors such as fitness, disease, and age. For example, Dishman et al. (2003) reported a step-wise increase in the magnitude of the change in a group of individuals ranging from low to high fit. Specifically, Dishman et al. (2003) reported a decrease of -0.46 ml/100ml/min in low fit men, compared to -0.73 ml/100ml/min in high fit men. Kinuyoshi et al. (2003) reported a decrease in forearm blood flow from -0.3 ml/100ml/min in obese, normotensive females to -0.8 ml/100ml/min in lean, normotensive females. The mean calf blood flow following a cold stimulus in the present study was 1.53 ml/100ml/min, which was -0.73 ml/100ml/min lower than resting arterial inflow. So, it appears the findings in the present study are somewhat consistent with the previous reports.

4.2 Physiological Pattern of Vascular Measures

A main objective of the present study was to assess vascular function using a variety of stimuli, rather than a single stimulus, to move toward a greater understanding of the factors that may be involved in the control of vasoconstriction and vasodilation. Moreover, the flow response to an integrated stimulus such as exercise may provide a further understanding of the interplay of the factors that control the distribution of flow. Thus, our quest was to assess vascular function in one visit, in which we would examine both vasodilation and vasoconstriction, and the integrated blood flow responses in the lower limb. To this extent, certain assumptions were used, such as the assumption that post occlusion reactive hyperemia tests vasodilatory properties of a region, that the cold pressor test is a test of vasoconstrictor properties, and that exercise provides a more integrated response.

Of critical importance in our study was the predictability of the responses and the stability and reproducibility over the course of visits. In regards to the predictability of the test, it was hypothesized that the pattern of the blood flow responses would be such that blood flow would increase significantly after lower leg occlusion, to an even greater extent after the bout of exercise, and significantly reduced as compared to baseline following the cold stimulus. The present study indeed confirmed this hypothesis, with virtually every participant behaving in the same manner. Given the predictability of these findings, several mechanisms can be discussed that could have accounted for the responses.

The mechanisms believed to explain the blood flow responses following lower leg occlusion are generally thought to involve metabolic and myogenic factors (Thijssen et al., 2005). Thijssen et al. (2005) reported that vasodilation following occlusion is attributable to local release of mediators and metabolites. In addition, Joyner's group concluded that inhibition of vasodilating prostaglandins reduces reactive hyperemia, while nitric oxide only plays a minimal role (Engelke et al., 1996). Therefore, it appears the most important contributors to the reactive hyperemic response are local vasodilators that are released from the tissue. The role of the myogenic response to the flow response after occlusion is believed to be secondary to the pressure release from occlusion, as the arteries react to a drop in the local blood pressure in the tissue region distal to the occlusion. It is thought that, under such conditions, smooth muscle relaxes, maximizing the available flow. It is perhaps important to further understand the time course of the controlling factors during the reactive hyperemic response. Bjornberg et al. (1990) reported that myogenic relaxation mechanisms exert their greatest influence on the flow

response if the occlusion phase is less than 30 seconds. In contrast, metabolic mechanisms are thought to be responsible for the majority of the flow response for occlusion times higher than 30 seconds. Arguably, the reactive hyperemic response in the present study is a combination of the two primary mechanisms, as the 5 minutes of occlusion is more than likely sufficient to generate substantial concentrations of metabolites which may act on the precapillary sphincters of the local tissue, whereas the myogenic response aids in the relaxation of smooth muscle initially, followed by a modulation of vasoconstriction/vasodilation with each pulse of high flow after occlusion. So arguably, the reactive hyperemic response seen is a combination of metabolic and myogenic influences in the region of interest. Also, in addition to the current study finding no differences between blood pressure and heart rate during rest and blood pressure and heart rate during occlusion, the previously mentioned studies reported no influence of a central role in blood flow control following occlusion.

In the design of this study we selected dynamic exercise as the second stimulus. The blood flow responses during dynamic exercise are thought to follow a rather distinct pattern. Initially, blood flow at the onset of exercise increases rapidly, followed by a second phase of a more gradual increase until the demand is met (Van Beekvelt et al., 2001). In the present study the major contributor to the short bout of exercise is thought to be the muscle pump and myogenic properties, with a smaller role for other mechanisms including local metabolite release, functional sympatholysis, and/or nitric oxide (endothelial-derived releasing factor).

The muscle pump is believed to contribute to 60% of the initial increase in blood flow following two seconds of exercise onset (Tschakovsky et al., 1996). In addition,

Tschakovsky et al. (2004) concluded using single contractions of different intensities that initial blood flow increase from one contraction may be due to rapid-acting vasodilatory mechanisms. Tschakovsky & Sheriff (2004) reinforced the beliefs that both the muscle pump and unknown vasodilatory mechanisms contribute to hyperemia within five seconds of exercise, while a different study concluded that an increase in blood flow due to sympatholysis begins at 30 seconds of continuous exercise (Tschakovsky & Hughson, 2003).

In summary, it is believed the mechanisms of greatest consequence in the current exercise bout is the muscle pump and myogenic properties, unknown vasodilatory mechanisms, and to a lesser extent functional sympatholysis. Thus, it would appear the exercise stimulus is indeed an integrated response of several important blood flow controllers.

The final vascular stimulus in this study was the cold test. As hypothesized, the cold stimulus provokes a decrease in blood flow from rest. The physiological mechanism causing this decrease is believed to be vasoconstriction, secondary to increased alpha adrenergic agonists. Previous work has reported increased blood pressure and heart rate during a cold stimulus (Koch et al., 2003), along with decreased blood flow to the peripheral vasculature (Dishman et al., 2003). The current study also presents an increase in heart rate during the cold stimulus when compared to pre-cold pressor test heart rate, along with a decrease in blood flow to the calf, which illustrates a change in central control factors. The rapid change in the autonomic nervous system during a cold stress increases the muscle sympathetic nerve activity in the body, which in turn may cause vasoconstriction in the muscles (Kinuyoshi et al., 2003). Our data presents a blood flow

decrease similar to previous studies (Dishman et al., 2003; Kinuyoshi et al., 2003) and a heart rate increase similar to previous studies (Kinuyoshi et al., 2003; Sevre & Rostrup, 1999), which we may attribute to the increasing autonomic nervous system during our cold stimulus.

The unique contribution of the present study is the combination of both vasodilation and vasoconstriction stimuli, in addition to an integrated vascular stimulus, on lower leg blood flow responses in one visit. The pattern was distinct with virtually all subjects responding in the same manner (i.e. the largest increase in flow after exercise and a reduction in flow after the cold test). Thus, it would appear that, with regards to the noticeable pattern of blood flow changes from rest, our protocol of measuring blood flow following different stimuli in one visit provides valuable evidence toward both the vasodilation and vasoconstriction properties of the vasculature, and an integrated response in the vasculature.

In addition to the stability of the resting blood flows between the stimuli, the flow responses were predictable. Other objectives of the study were to examine the pattern of the blood flow responses, the test-retest reliability between each individual stimulus and their respective individual measurements, as well as the reliability of the overall protocol of measuring the different stimuli in one visit. A univariate analysis of variance proved that, within one model, each stimulus was significantly different from the resting blood flow measures and the other stimuli ($p=.0001$), in addition to proving that there were no significant between-visit differences for the stimuli responses ($p=.989$). In evaluating the individual stimuli measurements, the correlation coefficients indicate fairly adequate test-retest reliability. For example, Alomari et al. (2003) reported a between visit correlation

coefficient of 0.81 for post-occlusion reactive hyperemic blood flow, while Mahankali et al. (1997) reported coefficients between 0.71 and 0.91 for post-occlusion reactive hyperemic forearm blood flow. Our lower leg reactive hyperemic blood flow (0.68, 0.56), exercise blood flow (0.6, 0.75, 0.71) and cold pressor test blood flow (0.59, 0.65) correlation coefficients did seem to be a bit lower than previously reported studies in the arm. However, the stability of our mean differences, along with finding no between-visit differences among the stimuli, indicates that our assessment of vascular function was fairly reliable between visits. The reliability of our protocol of measuring blood flow changes to all the aforementioned stimuli in one visit proved of significance [ICC=0.61 (95%CI = 0.307 – 0.81; P=.001)]. Ultimately, our demonstration of measuring blood flow following different vasodilator and vasoconstrictor stimuli in one visit may lead to a more comprehensive vascular study that will lead to the development of a vascular test. Such a test may lead to the identification of individuals at risk.

4.3 Correlations Between Blood Flow Measures

An important and rather unique finding in the present study was the associations between the vasoconstrictor responses, reactive hyperemia, and exercise flow responses found during one visit. More specifically, with greater reactive hyperemia blood flow responses, greater exercise blood flows and greater drops in blood flow following the cold stimulus were exhibited. These associations suggest that people who have a greater ability to vasodilate, also have a greater ability to vasoconstrict. It is this finding that is particularly intriguing. Generally, the greatest focus on understanding vascular function has been on the vasodilatory properties of the vascular system. However, it is clear from Guyton & Hall (2000) that the focus really should center on the distribution of blood flow

to and within muscle beds as it relates to understanding performance. Previous research has acknowledged that with age, limb blood flow is reduced due largely in part to chronically increasing sympathetic alpha-adrenergic vasoconstriction (Dinenno et al., 2001), with another study stating that there is an age-related decrease in the ability of vasculature to vasoconstrict during a cold stimulus (Feger & Braune, 2005). In addition, previous data from a study by Dishman et al. (2003) suggests that high fit men and women have a better ability to vasoconstrict than the low fit men and women, while it has also been reported that exercise training contributes to increased limb vasodilatory capacity to specific and non-specific exercising muscles (Boutcher & Boutcher, 2005). Therefore, with the current study correlating increased vasodilation with increased vasoconstriction, it may be possible for future research to find which areas of the vasculature benefit most from specific exercise training regimens with regards to specific populations. For example, Maeda et al. (2003) stated that older women increased vasodilation capacity due to three months of 30 minutes 5 days/week aerobic training reducing the endothelial vasoconstrictor, endothelin-1, concentration. Such interventions may advance exercise prescription recommendations for particular populations.

In summary, the associations found in our study, together with the pattern of blood flow responses, further illustrate the potential for development of a one visit vascular test that includes vasodilator and vasoconstrictor stimuli, which may have the potential to identify individuals at risk and advance exercise recommendations.

4.4 Clinical Relevance

The idea of the current study was, in one visit, to manipulate the vasculature using different stimuli to possibly learn more about vasodilator and vasoconstrictor properties

of the cardiovascular system. Most previous research has focused on only one property of the vasculature, using either a vasodilator or vasoconstrictor stimulus. It is important to understand blood flow distribution, a combination of vasodilation and vasoconstriction, as it relates to performance; therefore, we approached our study with this viewpoint, as it may open avenues for improved targeted treatments.

4.5 Limitations

As with every study, we remain cautious in our interpretations due to the inherent limitations associated with this type of study. One limitation of the current study may be a possible order effect. Our study consisted of 12 subjects, 10 of which were tested in the described protocol. However, in order to understand the order effect, we did manipulate the order for the remaining two subjects, and we found the same flow responses. Another limitation of our study is the assumptions of our vascular stimuli. Though in theory we may conclude that the aforementioned vasodilation, vasoconstriction, and other physiological mechanisms are responsible for the blood flow responses to the different stimuli, there is no certainty to our assumptions. Finally, one could question the exercise stimulus used in the current study, for we assumed 15 contractions of 60% MVC would give us a clear estimate of an integrated response. Therefore, future study may manipulate the duration, intensity, and even the mode of exercise stimulus used as it relates to integrated responses.

4.6 Conclusion

The purpose of this study was to assess vascular function using a variety of stimuli to potentially learn more about vasodilator and vasoconstrictor properties as they relate to blood flow distribution. The unique findings of this study were the patterned

response presented by the vasculature following the different stimuli and the correlations among our different vascular measures. The pattern we presented in this study illustrated occlusion as stimulating increased blood flow, exercise as stimulating the highest blood flow, and cold as stimulating blood flow lower than rest, while the correlations between the measurements demonstrated that with greater vasodilation properties, greater vasoconstriction properties are present. In addition, the stability and reliability of our vascular function measures were adequate. Though most previous research has focused on one property of vascular function, it is important to continue our view of studying blood flow distribution, which involves both vasodilation and vasoconstriction properties.

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