VELOCITY PROFILE OF ARTERIAL BLOOD FLOW AND SERVERITY OF PERIPHERAL

ARTERIAL DISEASE

by

HUI-JU YOUNG

(Under the Direction of Kevin McCully)

ABSTRACT

The flow mediated dilation (FMD) test assumes Poiseuille's Law to calculate the shear

stimulus. This universal estimation of shear stimulus could potentially lead to measurement error.

This study examined evidence for potential differences in shear stimulus and the time course of

reactive hyperemia in the femoral and brachial arteries in subjects with and without peripheral arterial

disease (PAD). The variability of femoral velocity at peak systole was greater in PAD compared to

controls (half range at rest: $p=0.006$; full range at rest: $p=0.03$; half range at peak: $p=0.02$; full range at

peak: $p=0.02$). The shape of velocity profile was not different between groups. Time course of

reactive hyperemia in the femoral artery was longer in subjects with PAD compared to the control. In

conclusion, the distribution of blood velocity across the vessel walls was similar in healthy and

diseased people, supporting the use of Poiseuille's Law in FMD studies.

INDEX WORDS: Reactive Hyperemia, Shear Stimulus, Velocity Profile

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DEDICATION

To my parents, Robert Young, Joy Chen, and my sister, Jackie Young. Without your support and love, I would not be the person I am today.

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CHAPTER 1

INTRODUCTION

Higher prevalence of cardiovascular disease over the past several years has lead to importance in measuring arterial function to predict cardiovascular events. Peripheral Arterial Disease (PAD), one of the major forms of vascular disease, has remarkably affected the quality of life in people who suffer from the disease. One of the prominent symptoms found in people with PAD is intermittent claudication (29). It is characterized by having leg pain during exercise due to blood flow insufficiency. This pain sensation could contribute to physical inactivity in people with PAD (3). Therefore, the need of diagnostic tests to detect early risk factors of PAD has raised the attention of many vascular scientists.

The vascular endothelium is believed to play a key role in maintaining vascular tone and vascular homeostasis (43). Endothelial dysfunction can lead to abnormalities in vessel dilation and is associated with coronary risk factors and cardiovascular events (15, 46). Doppler ultrasound has been widely used to examine vascular dilatory response and patterns of blood flow; therefore, it has been recognized as a valuable diagnostic tool for assessing endothelial dysfunction (41). One particular ultrasound method used to detect endothelial dysfunction is called flow-mediated dilation (FMD)(41). FMD is an endothelium-dependent, shear stress-induced dilatory response to a 5-minute ischemia produced by cuff occlusion. It has been proposed that FMD should be normalized with shear stimulus in order to express true FMD.

Poiseuille's Law states that shear rate is defined as 8* measured mean flow velocity divided by artery diameter (13). Based on this equation, a linear blood velocity (laminar flow) across arteries is

assumed. However, according to literature, when blood flow exceeds a certain velocity (Reynolds number), flow tends to become chaotic. Blood in the centerline of an arterial lumen flows with much higher velocity than the blood flow near the vessel wall, and the difference appears to be larger in response to higher resistance (i.e. plaque) of the vessel wall (34, 41). This indicates that the current estimation of shear rate with respect to Poiseuille's Law could potentially lead to measurement error.

Most studies have also examined the value of velocity and vessel diameter after reactive hyperemia. Very few studies, if any, have looked at the time course of reactive hyperemia comparing people with and without vascular disease.

Statement of the Problem

The calculation of shear stimulus assumes the same parabolic flow in every individual. This assumption of shear stimulus might be misleading since flow patterns could be altered by factors, such as the presence of plaque, vessel dilatory response, and many other reasons.
On the other hand, time course of reactive hyperemia and its association to PAD have not been fully studied.

Specific aims

This study has two aims. The first aim is to evaluate changes in flow velocity profile across the vessel wall in the arm and diseased leg by comparing two groups of people; one with evidence of mild to moderate arterial disease and the other without. The second aim is to evaluate the impact of PAD on the time course of reactive hyperemia, both in the arm and diseased leg..

Hypotheses

(1) The velocity profile will be more parabolic and the variability of velocity will be greater in people
   with PAD when compared to the control group.

(2) People with PAD will have a slower onset and recovery of reactive hyperemia when compared to the control group. Also, differences with PAD will occur both in the diseased leg and in the arm.

Significance of the Study

Currently, most vascular studies do not account for variability in velocity profile across arteries when calculating shear stress or shear rate. They have assumed velocity profile is parabolic and is under the same condition for every person regardless of the presence of vascular disease; however, it has been reported in literature that blood flow tends to become chaotic (turbulent flow) under conditions of plaque in arterial walls or increased viscosity of blood. This study seeks to investigate two new ways of measuring vascular disease in people with mild to moderate peripheral arterial disease (PAD) by examining the variations in blood velocity profile across femoral and brachial arteries, as well as the time course for velocity to reach its peak and to return to baseline after reactive hyperemia. Successful demonstration of these new measurements will hopefully be used as markers of early disease stages.

CHAPTER 2

REVIEW OF LITERATURE

Cardiovascular Disease & Peripheral Arterial Disease

Cardiovascular disease is one of the leading causes of morbidity and mortality in the world. According to the statistics in 2006, there were more than 80 millions people in the United States suffered from one or more forms of cardiovascular disease (CVD) (American Heart Association Website). Due to its high prevalence of death, identifying the risk factors contribute to CVD has been a major project for scientists and researchers.

Peripheral Arterial Disease (PAD), similar to the condition found in CVD, is characterized by having arterial plaque affecting many areas of the body, especially lower extremities (14, 31). The statistics in 2008 from American Heart Association showed that there were approximately 8 million people in the United States affected by PAD and have higher risk of morbidity. Non-modifiable risk factors such as ethnicity and increasing age, and modifiable risk factors such as smoking, diabetes, hyperlipidemia, hypertension, have been shown to associated with higher prevalence of PAD (5, 8, 18, 32, 35)

One of the major symptoms found in people with PAD is intermittent claudication (2). Intermittent claudication (IC) refers to the need of frequent stop from walking due to the sensation of pain on patients' legs (3, 40). This sensation is particularly strong when inadequate oxygen cannot be delivered to muscles during exercise due to reduced blood flow distal to the stenosis but should diminish with the cessation of exercise (3, 14).

There have been evidences suggest that about 12% of the adult population has PAD and the prevalence of PAD does not differ in gender (32). Moreover, when one reaches the age of 70 or over, the prevalence of PAD becomes 2 in every 10 elderly in the United States and increases with history of diabetes or smoking at the age of 50 or older (19, 32). As mentioned by McDermott (28), about 20 to 30 % of older patients' lower extremities are affected by PAD. The nature of this disease often leads to inadequate amount of physical activity, especially in people with severe PAD and in elderly, and could contribute to higher risk of heart problems, stroke, diabetes, and amputation (19). Therefore, finding better ways to detect early stage of PAD is important.

Diagnosis of Peripheral Arterial Disease

From the cheapest way, walking impairment questionnaire, to the high technology, magnetic resonance spectroscopy, there are several ways being used to diagnose PAD. Among them, one of the most frequently used tests is Ankle and Brachial Index (ABI) test. ABI, a non-invasive and a simple test, is done by taking the ratio of one's systolic pressure on the ankle to that on the arm while the person is in the supine position (19). In healthy, normal individuals the systolic pressure is slightly higher in the leg than in the arm, giving a ratio of 0.9 to 1.4 (19). A person with an ABI ratio lower than a 0.9 indicates that blood flow to the lower extremity is reduced and is considered to have PAD (19, 32). One limitation that ABI test has is the inaccurate reading of systolic blood pressure when measurements are done on a non-compressible vessels as observed in people with diabetes and can lead to misinterpretation of the disease (32).

Progressive walking test (PWT) is another diagnostic test for PAD. This test is generally done by having patients slowly walk on a treadmill until the need to stop walking due to claudication (26). Symptoms free walking time and maximal walking time are recorded. The use of walking tests to assess PAD has advantages since it can potentially locate the stenosis by where the pain occurs.

However, as M.M. McDermott et al. reported in her study, 63% of subjects with PAD had no symptom of claudication (27). This suggests that the progressive walking test should not be used alone for diagnosing PAD.

The other diagnostic tool is Duplex ultrasonography. Ultrasonography has been widely used to examine vascular health and can provide information about the location and severity of stenosis. Due to its non-invasive and accuracy, many tests have been develop utilizing ultrasound to assess vascular diseases, specifically, to detect endothelial dysfunction (need reference).

The Endothelium and its dysfunction

The endothelium is the thin layer of cells that can be found on the inner surface of blood vessels and is believed to play an active role in maintaining vascular tone and in regulating vascular homeostasis and reactivity, through the release of several vasodilators (9, 20, 45). Among the vasodilators, nitric oxide (NO) has been greatly studied due to its function in preventing atherosclerosis; therefore, a reduction in NO's bioavailability is believed to play a part in development of vascular disease (38). The dysfunction of endothelium can lead to decreased expression of NO, and therefore, cause abnormal dilatory responses and is the underlying cause of plaque build-up. According to Alam et al. (1), diseases such as diabetes mellitus, hypertension, systemic and sclerosis have been found to be associated with endothelial dysfunction. Also, previous studies have reported that endothelial dysfunction occurs prior to the formation of atherosclerosis (7, 39, 44), this suggests the importance of detecting endothelial dysfunction.

Flow-mediated Dilation and Reactive hyperemia

Flow-mediated dilation (FMD) test has been proposed as a non-invasive method and is a commonly used technique to detect endothelial dysfunction (37). Originally proposed by Celermajer et al., this technique creates tissue ischemia by 5-minute cuff occlusion distal to target arteries (i.e.

brachial artery and femoral artery). Upon the release of cuff, a sudden increased blood velocity (reactive hyperemia) acts as a shear stimulus that causes a dilatory response of the arteries[7, 37]. This resulting response is called flow-mediated dilation (FMD). This particular FMD was found to be NO dependent and was believed to be strongly correlated with coronary artery function[2, 23]. According to Hayoz et al. (16), subjects with congestive heart failure (CHF) had more than 40 seconds delayed time for diameter to reach its peak value and was possibly due to endothelial dysfunction. They also suggested that reactive hyperemia appeared to be abnormally lower in patients with CHF.

Reactive hyperemia, as mentioned above, is a sudden increase of blood flow in response to ischemia produce by cuff occlusion. Evidences showed that the pattern of flow velocity after reactive hyperemia in subjects with vascular disease was different from normal, healthy subjects. Shepherd (22) observed that the immediate post-exercise flow was significantly lower in subjects with arteriosclerosis and the blood flow appeared to return the baseline with a much slower rate. Although it was not emphasized in the paper, it was suggested that longer time was required for blood flow to reach its peak velocity as the severity of disease increased.

Blood flow & Shear Stimulus

Continuous blood flow in a long straight vessel is believed to flow parallel (laminar flow) with a maximum flow at the center and minimum flow at the vessel walls. This pattern of blood flow is called parabolic flow or parabolic velocity profile which describes the shape of flow velocity at an instantaneous time (13). Reynolds number is defined as the ratio of inertia forces to viscous forces and is used to describe the flow pattern of fluids. Under conditions when blood velocity becomes too fast (larger Reynolds number is reached), flow becomes turbulent. Unlike laminar flow, turbulence is a non-parallel, chaotic flow which increases the pressure required to drive a given flow and can happen when blood travels in narrowed region (sites of stenosis), as stated by Continuity rule[13].

Evidences have supported that it is necessary to normalize the dilatory response to shear stress or shear rate in order to present the true FMD (38). Shear stress, as stated by Poiseuille's Law, is equal to 4 times the product of blood viscosity and measured flow divided by the product of pi and the third power of lumen radius (13). Due to the more invasive measure of blood viscosity, shear rate, defined as 8 times measured mean blood flow velocity divided by the lumen diameter, is more frequently used rather than shear stress (13, 34). A study conducted to examine if peak or total reactive hyperemia determined the magnitude of FMD used the average maximum blood velocity of every 3-second to calculate shear stress and to establish profile of blood flow velocity (37). Kooijman et al. (23) manually recorded blood velocity from the flow velocity integral (FVI) every five seconds from 15 to 45 seconds post-cuff deflation and the data was used to calculate mean blood flow and mean wall shear rate.

However, as indicated by Parker et al. (34) and Kremkau (13), Poiseuille's Law assumes blood flows in perfect, straight arteries with steady uniform flow which can be hardly observed in vivo. This suggests that Poiseuille's Law introduces unavoidable error and should only be considered as a best estimate of true shear rate (34). As described above, most studies have assumed the flow velocity was linear across arteries without taken the variability in velocity profile into account. This might be due to unavailable technology to measure the variability. In fact, literatures and studies have qualitatively indicated that the potential variation of blood velocity across an artery. Both Kremkau (13) explained the variability of blood velocity profile using graphs and Doppler spectra. Cavalcanti & Carota [6]recognized the importance to examine the variability of blood velocity at the sites of stenosis. They evaluated velocity profile using 3-D view and found that the shape of flow velocity profile should be considered when analyzing blood velocity. Moreover, Hoskins concluded that overestimation or underestimation could occur when using maximal blood velocity or mean blood velocity across

arteries as representation of non-linear blood flow to calculate shear stress[6]. To date, there are few

quantitative evidences, if any, describe the variability in flow velocity profile.

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CHAPTER 3

VELOCITY PROFILE OF ARTERIAL BLOOD FLOW AND SEVERITY OF PERIPHERAL

ARTERIAL DISEASE

Hui-Ju Young

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Abstract

Flow mediated dilation (FMD) has been used to characterize cardiovascular health in a wide variety of clinical conditions. A key assumption to this test is the use of Poiseuille's Law to calculate the shear stimulus. This "one-flow pattern fits-all" estimation of shear stimulus could potentially lead to measurement error. The first aim of this study was to examine the variability of flow velocity in subjects with and without peripheral arterial disease (PAD) by analyzing spectral and color Doppler at rest and during peak reactive hyperemia, in both femoral and brachial arteries. The second aim of this study was to compare the time course of reactive hyperemia in femoral and brachial arteries between the healthy and PAD groups. A significant difference was found when comparing the variability of femoral velocity at peak systole in the subjects with PAD to the control group (half range at rest: $p = 0.006$; full range at rest: $p = 0.03$; half range at peak: $p = 0.02$; full range at peak: $p = 0.02$). A similar shape of flow velocity profile in the brachial artery was observed between groups (area outside the curve: 23 (4.0) % in PAD; 24 (3.4) % in control; $p = 0.6$). Time course of reactive hyperemia in the femoral artery was found to be significantly different as well between groups (time to peak velocity: 45.8 (16.8) seconds in PAD; 12.0 (3.2) seconds in control; $p = 0.004$). In conclusion, the distribution of blood velocity across the brachial vessel walls appeared to be similar in healthy and diseased people, supporting the use of Poiseuille's Law in FMD test on the brachial artery. However, PAD was associated with increased blood flow variability and a delayed blood flow response to reactive hyperemia, only in the diseased leg.

Keywords: Reactive Hyperemia, Shear Stimulus, Velocity Profile

Introduction

Cardiovascular disease is a major cause of mortality and morbidity. Peripheral arterial disease is a common variant of cardiovascular disease, occurring in approximately 12% of adult the population (17, 32). Understanding the physiological basis of the clinical symptoms associated with PAD have been difficult because of multiple factors, such as vessel occlusion due to plaque formation, endothelial dysfunction, and arterial stiffening, that influence vascular function (32). Previous studies have suggested that PAD is associated with increased turbulence in blood flow (13, 47) and delayed flow kinetics (22). The need for tests to detect early risk factors has been brought to the attention of many vascular scientists and researchers.

The brachial-artery flow-mediated dilation (FMD) test is a commonly used approach to measure the health of blood vessels(45). FMD test is the percent change in arterial diameter in response to reactive hyperemia caused by 5 minutes of ischemia (34). This method has been shown to indicate nitric oxide bioavailability and to be an early predictor of cardiovascular disease (9, 15, 38, 41). Several studies have pointed out the need to consider initial arterial diameter as well as blood velocity when measuring FMD. One of the ways of determining the reactive hyperemia stimulus is to calculate 'shear stimulus' using Poiseuille's law (34, 38). The key assumption of shear stimulus is that the relationship between arterial blood velocity at the lumen center and the shear stimulus at the endothelial cell wall is the same for every individual. Given the potential influence of arterial disease on blood velocity across the vessel wall, this assumption needs to be tested.

The purpose of this study was to evaluate changes in blood velocity across the vessel wall using Doppler ultrasound in the arms and legs by comparing people with and without PAD. We also evaluated the impact of PAD on the time course of reactive hyperemia response to five minutes of ischemia. It is hypothesized that the variability of flow velocity at a point of time is larger in people

with PAD when compared to individuals without the disease; moreover, the velocity profile during systole is different when comparing the two groups. It is further hypothesized that the time course of reactive hyperemia is different in people with PAD.

Methods

Study Subjects

Subjects were between the ages of 55-85 years old, with and without the evidence of mild to moderate peripheral arterial disease. Recruitment was performed through newspaper ads, word of mouth, and vascular clinics. This study was approved by the University of Georgia Institutional Review Board and informed consent was obtained from all individuals before data collection and after a detailed description of the study was provided to them.

Subjects with advanced diabetes, heart failure, and any known medical condition that would be unsafe for them to participate in the study and subjects who were enrolled in any competitive exercise/sport program were excluded. Subjects were determined to be in the PAD group by three measurements: Ankle and Brachial Index (ABI) test, ultrasound test, and progressive walking test. Subjects with PAD who could not walk for more than 30 seconds or who ABI less than 0.4 were excluded as well. All subjects, except subjects recruited for reproducibility test, were interviewed for their general health statue with a Health Screening Interview (HIS) form and filled out a Walking Impairment Questionnaire (WIQ) prior to the experimental tests in the first session to determine their eligibility.

Experimental Design

All subjects were asked to make two test sessions that conducted in the Vascular Physiology Laboratory, Kinesiology Department. Both sessions lasted 90 to 120 minutes depending on which day

the progressive walking test was performed. The first session consisted of informed consent, Health Screening Interview (HSI), Walking Impairment Questionnaire (WIQ), Ankle and Brachial Index (ABI) test, and an ultrasound test. The second session consisted of an ultrasound test. A progressive walking test was conducted in either the first session or the second session depending on the time availability of the subjects and of a cardiologist who supervised the walking test.

Each subject was scheduled to come for his/her two sessions maximally a week apart and at the same time of day unless their schedule didn't allow them to. All subjects were asked to not exercise, not ingest caffeine, high fat goods, and Vitamin C, and to not smoke for at least 4 to 6 hours before their session. They were asked to just have a regular meal or a light snack before they came to their sessions.

Experimental Procedure

Ankle and Brachial Index Test

Ankle and Brachial Index (ABI) test was used to indicate the presence of mild to moderate PAD(30). Subjects were instructed to rest (supine) on an exam table for 10 minutes. A small ultrasound probe (the size of a thick pencil) was used to hear blood flow in the radial artery in the arms and the posterior tibial and doris pedis arteries in the legs. Velocity waveforms were recorded for quality assurance, and then the systolic blood pressure was recorded for all three arteries, both right and left sides. Subjects who had ABI less than 0.9 were in the PAD group and subjects with ABI higher than 0.9 were in the control group. Ultrasound test was done on the leg with the lowest ABI value for both PAD and control groups.

Ultrasound Measurement

BLOOD FLOW VELOCITY. All subjects were asked to rest supine for at least 10 minutes or until stable baseline velocity was obtained. For the first session, measurement of blood velocity was

made on both femoral and brachial arteries. A blood pressure cuff was positioned below the knee (with lower ABI) or same side below the elbow. A GE 400CL duplex color Doppler imager (GE Medical) was used in this study. Ultrasound CF mode (color flow image) was used to locate femoral and brachial arteries. Color scale was adjusted to avoid aliasing artifact and two color images were saved for later analysis. Ultrasound PW-mode (spectra Doppler images) was used for velocity measurement. Cuff was inflated to 100mmHg above subjects' systolic blood pressure for 5 minutes to induce reactive hyperemia. The gate (ultrasound window) of the ultrasound measurements was set across the entire vessel (large gate) so that the entire range of velocities in the artery could be recorded (see figure 3.1). Baseline measurement was made until stable velocity was obtained and velocity during cuff inflation was measured. Velocity measurements after cuff deflation were recorded continuously for 5 to 8 minutes or until blood flow returned to baseline. Data was collected in 15-second segments and was stored for future analysis.

For the second visit, same cuff-inflation procedure was performed twice on the femoral artery. The velocity window of the ultrasound measurements was set to 1 mm (small gate) and was placed in the center of the vessel lumen (see Figure 3.2). The order of measurement on the vessel wall and the center of the vessel were randomized. Two CF mode images were recorded for reproducibility purpose.

Progressive Walking Test

A licensed cardiologist, supervised the progressive walking test. All subjects performed a progressive walking test at either the first or second session, depending on the subjects' and physician's availability. The subjects were asked to wear comfortable walking clothes and shoes. ECG electrodes were placed on the chest to record electrical heart activity and to monitor cardiac abnormalities. The entire test was 12 minutes in duration. All subjects were asked to walk at 2 mph

without supporting their body weight with their hands. The grade of the treadmill was increased by 2% every two minutes. The subjects were constantly being asked how their legs felt and were required to report the time and location when leg pain first occurred if there was any. All subjects were encouraged to walk as far as they could but to stop if their legs hurt too much. Symptom (pain) free walking time and maximal walking time were recorded. The subjects were monitored until they had fully recovered from the walking.

Data Analysis

Range of Flow Velocity

All analysis was done using 15-second videos of Doppler spectra obtained with either a large gate or small gate at centerline in the femoral artery and a large gate in the brachial artery (see Figure 3.1, 3.2 &3.3). In order to optimize visualization on spectral pixel, the speed width of Doppler spectra was increased until only one cardiac cycle was covered on the ultrasound screen. Gain was adjusted to minimize artificial pixel noise. The selected cardiac cycle was saved as JPEG images and was analyzed using ImageJ. A Profile of pixel distance and intensity was obtained by drawing an analytical line across at peak systole of the selected cardiac cycle (see Figure 3.3 A&B). Pixel distance was calibrated with the velocity scale on the ultrasound machine. Half range from the peak velocity and full range velocity were calculated to represent variability of velocity. Same procedure was followed for each subject.

Velocity Profile.

Color flow videos were played frame by frame on the ultrasound machine to check aliasing and selected frames during systole were saved jpeg images. Frames were analyzed using ImageJ to obtain pixel distance and pixel intensity. Area under the curve was calculated and subsequently subtracted with 100% to obtain the percent of area outside the curve (see Figure 3.4 A&B). The % of

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area outside the curve was used to represent the flow velocity profile. The same procedure was followed for each subject.

Time Course of Reactive Hyperemia

15-second videos of blood velocity were recorded at rest and during peak reactive hyperemia. Time averaged mean velocity (TAMEAN), time averaged max velocity (TAMAX), and acceleration were obtained from the built-in velocity analysis software that is included within the ultrasound machine. Time course of reactive hyperemia was established using TAMEAN. Resting velocity, time to peak velocity, peak velocity (TAMEAN, TAMAX, and acceleration), and time to half returned velocity were recorded for each subject (see Figure 3.5). Data that had weak signal and couldn't be analyzed was withdrawal from analysis.

Arterial Diameter

Arterial diameter was determined by using customized software. 15-seconds videos that were saved on the ultrasound hard drive were transferred to a computer where the videos were decompiled into individual frame (jpeg) for analysis. Arterial diameter was used to calculated blood flow and shear stimulus.

Blood Flow

Blood flow during resting and during peak reactive hyperemia was calculated using vessel radius^2 * velocity.

Shear Stimulus

Shear stimulus was calculated using 8* TAMEAN velocity / vessel diameter. Area under the curve (AUC) to the peak reactive hyperemia and to 60 seconds after the release of cuff was then calculated. This calculation was done with the assumption that blood viscosity does not differ substantially between subjects.

Statistical Analysis

Data are presented as mean and standard deviation for each variable. Time course of reactive hyperemia, range of flow velocity (half range and full range), and velocity profile between the two groups were compared using t-test. Each hypothesis was accepted if level of significance was below 0.05. No correction was made for multiple comparisons. Correlations were calculated between variables: ABI, symptom-free walking time, time to peak velocity, and time to half returned velocity.

Result

<u>*Study Subjects*</u>

A total of 21 subjects were enrolled in the study. Table 3.1 shows the physical and health characteristics of the study subjects. There were 6 subjects (1 female & 5 males; mean age ± SD = 73.4 ± 9.1) in the PAD group and 14 subjects (9 females & 5 males; mean age ± SD = 65.1 ± 9.4) in the control group. Three subjects didn't complete the second session due to unavailability. One subject's data was excluded from data analysis due to a graft on the brachial and femoral arteries.

One of the six subjects in the PAD group (14%) had Diabetes. All subjects (100%) in the PAD group had family history of vascular disease and all of them suffered from heart disease. Five of the six subjects (71.4%) in the PAD group had hypertension and were on medication. One subject (14.3%) in the PAD group was an active smoker. In the control group, eight out of fourteen subjects (57.1%) had hypertension and were on medication. Three control subjects (21.4%) had heart disease. None of the control subjects had diabetes and one subject was an active smoker (7.1%).

The results of Walking Impairment Questionnaire, Ankle and Brachial Index (ABI) test and

Progressive Walking Test are presented in Table 3.2. All subjects completed the walking

questionnaire and ABI test. Three subjects did not perform the walking test due to time restraints and

one subject was unable to perform the walking to test due to temporary reliance on the walker. Two

subjects in the PAD group reported claudication during the walking test but were able to complete the

entire 12-minute test. One subject stopped the walking test at 3 minutes 58 seconds due to shortness of

breath. This data was considered invalid and, therefore, was not included in the data analysis.

Range of Doppler Spectra at Peak Systole

Range of Doppler spectra at peak systole in the femoral and brachial arteries is presented in

Figure 3.6, 3.7, and 3.8. The range of Doppler spectra at peak systole in the femoral artery measured

with large gate was significantly different between groups, both at rest and at peak reactive hyperemia

(half range at rest: $p = 0.006$; full range at rest: $p = 0.03$; half range at peak: $p = 0.02$; full range at

peak: $p = 0.02$). The PAD group had a larger Doppler spectra range at peak systole when measured

using small gate at centerline, when compared to the control group (PAD half-range: $39 \pm 11.8\%$;

control half-range: $30 \pm 6.4\%$; PAD full-range: $75 \pm 22.7\%$; control full-range: $58 \pm 12.9\%$); however,

the difference was not significant ($p = 0.2$). The range of Doppler spectra at peak systole in brachial

artery were not significantly different between two groups (see figure 3.8).

Color Image Analysis

Color image analysis for both femoral and brachial arteries is presented in Figure 3.9 displays

a comparison of flow velocity profiles of the femoral and brachial arteries between PAD and control

groups. Based on the area-outside-the-curve calculation, the differences in velocity profile of the

femoral artery between PAD and control groups, was not statistically significant. Similarly, the shape of velocity profile between PAD and control group brachial arteries was not statistically significant.

Blood Velocity & Time Course of Reactive Hyperemia

Velocity and time course during the reactive hyperemia for each subject are presented in Table 3.4. Resting femoral diameters were not significantly different between groups (PAD: 0.6 ± 0.1mm; control: 0.6 ± 0.1mm; $p = 0.97$). Resting brachial diameters were found to be slightly smaller in the control group but the difference between groups was not significant (PAD: 0.5 ± 0.1mm; control: 0.3 ± 0.1mm; $p = 0.08$). Femoral flow velocity (PAD: 7.4 ± 6.8cm/s; control: 8.0 ± 3.0 cm/s) and brachial flow velocity (PAD: 7.4 ± 6.8cm/s; control: 8.0 ± 3.0cm/s) at rest were not significantly different (femoral: $p = 0.8$; brachial: $p = 0.9$). There was no significant difference in femoral artery blood flow between PAD and control groups. There was no significant difference in brachial artery blood flow between PAD and control groups.

Figure 3.10 shows a representative graph of peak hyperemia time course of reactive hyperemia in femoral artery between two subjects, one with PAD and one without PAD. Time to peak velocity in femoral artery showed a significant difference between groups (PAD: 45.8 ± 16.8s; control: 12.0 ± 3.2s; $p = 0.004$). There was also a significant difference in time to half-returned velocity between groups (PAD: 79.0 ± 27.7s; control: 30.5 ± 14.6s; $p = 0.006$). Subjects in the PAD group showed approximately 2 times lower peak TAMEAN velocity (PAD: 26.8 ± 14.4cm/s; control: 50.9 ± 13.2cm/s; $p = 0.007$) and about 1.5 times lower TAMAX velocity (PAD: 47.8 ± 26.9cm/s; control: 78.8 ± 19.9cm/s; $p = 0.037$) when compared to the control group. A representative peak hyperemia and time course of reactive hyperemia in brachial artery is presented in figure 3.11. Time to peak velocity, time to half returned velocity, and peak velocity (TAMEAN &TAMAX) in the brachial artery were not significantly different between groups. Slightly larger

diameter of brachial artery was found in the PAD group but the difference between groups was not

significant (PAD: 0.5 ± 0.1mm; control: 0.3 ± 0.1mm; $p = 0.08$).

Discussion

The main finding of the present study was that there was no significant differences in flow

velocity profile across the vessel wall between subjects with and without PAD. This was contrary to

our hypothesis, as we thought that the subjects with PAD would have significant differences in

velocity from the center of the vessel to the wall (nonlinear). Few previous studies have addressed

this issue. Atkinson et al. (4) investigated reliability of normalizing FMD to shear stimulus and

concluded that the relationship between shear stimulus and FMD may not be linear and the

normalization might not be applicable to every individual. Duivenvoorden et al. (11) recognized the

potentially oversimplified calculation of shear stimulus and developed a MRI protocol to estimate

shear stimulus with blood velocity near the arterial wall.

In the current study, two novel methods were used to compare the flow velocity profile between

groups. The first one used a comparison of variability of Doppler spectra measured with a large gate

and a small gate, during rest and at peak reactive hyperemia. The comparison was done with half

range and full range calculations. The second method used color flow images to compare the velocity

profile during systole between groups. Measurements were made at rest and after reactive hyperemia.

We hypothesized that the increased speed of the blood during reactive hyperemia would enhance the

potential differences between subject groups. However, we basically found the same results with both

conditions.

There was a significant difference in velocity variability in the femoral artery between groups with large-gate measurement, and this finding agrees with previous literature. In F.W. Kremkau's diagnostic ultrasound textbook (13), a broader Doppler spectra (larger variability of flow velocity) were presented in people with vascular disease and were believed to be associated with turbulent flow due to the presence of stenosis. A greater significance was observed with the half range calculation over the full range calculation, thus suggesting the half range method was more sensitive in capturing the variability of velocity between groups. Additionally, this study observed a trend towards the association of lower ABI values and higher variability of velocity (see figure 3.12). This suggests that the variability of flow velocity in the femoral artery during rest could potentially be a useful indicator of disease severity. A larger sample size and subjects with a wider range of ABI values are needed to further investigate this relationship. The variability of velocity during rest, measured with a small gate at centerline, showed a non-significant difference between groups. When compare the variability of velocity measured with large gate to that measured with small gate, the variability of velocity measured with small gate seems to have the same pattern as the one measured with large gate although the small gate measurements were not significantly different between groups. Small sample size could have contributed to this non-significance. If the variability of velocity at the lumen center was truly different, it suggests that the significant difference found in the femoral variability of velocity measured with large gate might be due to the variability of velocity in the enter of lumen and was not because of differences in velocity profile. The analysis f velocity profile in the femoral artery using color images was not observed to be significantly different between groups. However, there was an observed difference between groups and the velocity profile in the PAD group was highly variable. This suggests that this analysis on the femoral artery might be underpowered and is inconclusive. Larger sample size will be needed to make further conclusion. On the other hand, in the brachial

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artery, there was no difference in variability of velocity. Unlike the leg, the range of Doppler spectra at peak systole in the brachial artery were not significantly different between the two groups and this non-significant difference was observed with both half range calculation and full range calculation. A similar velocity profile analyzed using color images was found in the brachial artery between groups. Since most FMD test is performed in the arm, these results suggest that the 'one flow-pattern fits all' approach with using Posiuelle's law to estimate shear stimulus maybe applicable to the arm (38).

The second main finding of this study was that there was a delayed time course of reactive hyperemia in the femoral artery in subjects with PAD. This had been shown in a previous study. Shepherd (22) examined the magnitude of blood flow in the calf after ischemic exercise in subjects with PAD and observed that the subjects with PAD had lower reactive hyperemia when compared to the control. Although it was not the main focus of his paper, the time course of blood flow after the exercise was observed to be longer. In the current study, a delayed time course was only found in the diseased legs, and not in the arms of the people with PAD. Proctor et al. (36) investigated potential differences in vascular reactivity between the arms and legs and recommended that generalization of vascular function in the forearm with the one in the lower extremity should be avoided. It was observed that the time to peak velocity during reactive hyperemia in the PAD group was approximately 4 fold slower than the control group and the time to half returned velocity in the PAD group was about 2.5 fold slower than the control group. Time-averaged-mean (TAMEAN) velocity at peak reactive hyperemia was about 2 fold lower in the PAD group when compared to the control group. Time-averaged maximum (TAMAX) velocity during peak reactive hyperemia in subjects with PAD was about 1.5 fold slower than subjects in the control group. These findings are consistent with Shepherd's (22). On the other hand, there was no significant difference of time to peak velocity and time to half returned velocity observed in the brachial artery when comparing the two groups. These

findings suggest that PAD might only affect arteries in the lower extremity but not in the upper extremity, and the abnormality of the time course might be due to the impaired function of oxygen delivery to the lower extremity. Also, lower ABI values were observed to be associated with longer time to peak velocity in this study (see figure 3.13). The time course of reactive hyperemia in the femoral artery might serve as an indicator of PAD disease severity. However, larger sample size and subjects with a wider range of ABI values are needed to examine this relationship.

We found that the variability in velocity was greater in subjects with PAD than subjects in the control. This finding agrees with the previous result found in variability of velocity analysis and suggests the presence of turbulent flow in subjects with PAD even at rest. This was only true in the diseased legs and not in the brachial arteries. As observed in this study, some subjects with PAD had plaque along the entire vessel and making it difficult to avoid plaque while measuring velocity. This could potentially alter the flow pattern (turbulence caused by on-site plaque). This does suggest that the increased range of velocities is related to the plaque buildup in the diseased limb and not a function of impaired arterial function of blood chemistry. Blood viscosity was not measured in this study and could contribute to the study limitations. Although previous studies have suggested that the effect of individual variation on blood viscosity on shear stress is not significant (33), it should be noticed that most studies which looked at the effect of blood viscosity on shear stress were done on young, healthy individuals. In contrast, most of the subject in this study had at least one type of cardiovascular disease. Several studies have observed an elevated blood viscosity in patients with hypertension, diabetes, obesity, and hyperlipidemia when compared to the healthy control group(24). It is possible that subjects with PAD have higher blood viscosity which altered and prolonged their time to peak velocity during reactive hyperemia.

Since some subjects in the PAD group had a history of cardiovascular disease (CVD), statistics was performed to compare subjects in the control group with and without CVD. This was done in order to test if cardiovascular disease played a role in the difference we found in femoral artery reactive hyperemia. No significant difference was found between groups, this again could suggest that prolonged time-to-peak velocity in femoral artery might be a specific condition found in people with PAD.

Limitation

A potential limitation of this study was that the two sessions for some subjects were not conducted at a similar time of day due to the subjects' time constraints. As reported by Jones et al. (21), FMD was observed to be different when comparing repeated tests done in the morning and in the afternoon. This suggests possible alternation of blood velocity due to differences in dilatory response when tests are performed at different times in the day.

Another potential limitation was that some measurements of blood velocity were not kept below an angle of 60 degree. As reported by Thijssen et al. and several other literature (13, 41), measuring blood velocity with an angle more than 60 degree could increase the inaccuracy of estimation exponentially. Keeping the angle below 60 degree was found to be difficult on some subjects, especially on subjects who had thicker fat tissue. The angle on some subjects in this study was not kept below 60 degree throughout the reactive hyperemia test due to the diminishment of the arteries after compression or the movement of the subjects. Fortunately, data that did not have the right angle kept during the test could be adjusted afterward on the ultrasound machine, but measurement error with the angle above 60 degree should be taken into consideration. Diastolic blood pressure was not measured to calculate arterial compliance. Although the actual mechanisms have not been fully described, it is believed that blood velocity could be influenced by arterial stiffness. Also,

breathing might influence blood flow velocity, as observed in this study. Future studies will need to address these effects.

<u>Conclusion</u>

In conclusion, this study showed that in the femoral artery, there was a larger variability of velocity and slower time course of reactive hyperemia in subjects with PAD when compared to the control group. Further investigation is needed to examine the non-significant difference of velocity profile in the femoral artery between groups observed in this study. Moreover, variability of velocity and time course of reactive hyperemia was observed to be associated with ABI values and thus could be potential indicators of peripheral arterial disease severity. Perspective studies are necessary to examine the consistency of this finding. In the brachial artery, there was no significant difference in the variability of velocity and time course of reactive hyperemia observed between groups. This suggests that the assumption of Poiseuille's lab is acceptable when brachial artery is examined.

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Figure Legends

Fig 3-1: Representative Doppler ultrasound image of femoral artery measured with large gate.

Fig 3-2: Representative Doppler ultrasound image of femoral artery measured with small gate (1mm) at centerline.

Fig 3-3: (A) Analysis of range of Doppler spectra at peak systole (flow velocity variability). (B) Using ImageJ to obtain velocity (pixel)-intensity profile. First red line represents half range; second red line represents full range.

Fig 3-4: (A) Analysis of color image during systole. (B) Obtaining velocity profile from ImageJ and calculating the area under curve. Area outside the curve was calculated after normalize pixel distance and intensity to 100%

Fig 3-5: Representative excel scatter graph showing the analysis of time course of reactive hyperemia. TAMEAN, time-averaged mean.

Fig 3-6: Representative graph of the variability of velocity in femoral artery with large gate at rest and at peak reactive hyperemia.

Fig 3-7: Representative graph of the variability of velocity in femoral artery with large gate and with small gate at centerline at rest. LG, large gate; SG, small gate.

Fig 3-8: Representative graph of the variability of velocity in brachial artery with large gate at rest and at peak reactive hyperemia.

Fig 3-9: Representative graph comparing the velocity profile in femoral artery and in brachial artery between groups.

Fig 3-10: Representative graph comparing reactive hyperemia and time course of reactive hyperemia in the femoral artery between groups. TTP, time to peak; 1/2RT, half relaxation time.

Fig 3-11: Representative graph comparing reactive hyperemia and time course of reactive hyperemia in the brachial artery between groups. TTP, time to peak; 1/2RT, half relaxation time.

Fig 3-12: Representative graph showing the relationship between variability of velocity and the severity of disease measured with ABI.

Fig 3-13: Representative graph showing the relationship between variability of velocity and the severity of disease measured with ABI.

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Table 3.1 Subject Characteristics

Subject Characteristics		PAD Group (n = 6)	Control Group (n = 14)
Age, yrs		72.5 (9.6)	65.1 (9.4)
Gender		M = 5; F= 1	M= 9; F= 5
Race	Caucasian	6	12
	African American	0	1
	Asian	0	1
Height, cm		177.0 (9.6)	171.6 (6.5)
Weight, lbs		194.2 (46.9)	185.6 (51.4)
Family History of CVD, %		100	43
Hypertension, %		71	57
Known Coronary Disease, %		100	21
Diabetes mellitus, %		14	0
Active Smoker, %		14	7

PAD, peripheral arterial disease; CVD, vascular disease. Values are means (SD).

Table 3.2 Results of WIQ, ABI, and PWT

		PAD (n = 6)	Control (n = 14)	*p* Value
WIQ Score		64 (17)	57.4 (17.9)	0.1
ABI				
Worst	**PT**	0.76(0.16)	1.15(0.15)	0.001*
	DP	0.67(016)	1.01(0.14)	0.002*
Best	**PT**	0.94(0.08)	1.20(0.13)	<0.0001*
	DP	0.83(0.06)	1.20(0.25)	<0.0001*
PWT				
sfwt	**min**	3.88 (4.0)	12	0.004*
mwt	**min**	9.23 (2.8)	12	0.063

WIQ, walking impairment questionnaire; ABI, ankle and brachial index; PWT, progressive walking test; PT, post-tibial artery; DP, dorsalis pedis; SFWT, symptom-free walking time; MWT, maximal walking time. Values are means (SD).

Table 3.3 Range of Doppler Spectra at Peak Systole

			PAD	Control	p Value
At Rest	**Femoral**	**Large gate**			
			(n=6)	(n=12)	
		Half range, %	43 (5.3)	34 (5.3)	0.006*
		Full range, %	88 (18.9)	66 (10.0)	0.03*
		Centerline, small gate			
			(n=4)	(n=10)	
		Half range, %	39 (11.8)	30 (6.4)	0.2
		Full range, %	75 (22.7)	58 (12.9)	0.2
	Brachial	**Large gate**			
			(n=6)	(n=14)	
		Half range, %	40 (7.8)	40 (4.4)	1.0
		Full range, %	79 (8.7)	79 (8.7)	1.0
At Peak	**Femoral**	**Large gate**			
			(n=6)	(n=11)	
		Half range, %	50 (6.6)	41(7.5)	0.02*
		Full range, %	98(13.0)	80(14.8)	0.02*
	Brachial	**Large gate**			
			(n=6)	(n=12)	
		Half range, %	44 (5.8)	41 (7.6)	0.4
		Full range, %	86 (13.0)	82 (14.6)	0.5

Values are means (SD).

Table 3.4 Results of Time Couse of Reactive Hyperemia

	Femoral			Brachial		
	PAD	**Control**	*p-*value	**PAD**	**Control**	*p-*alue
Resting Characteristics						
Diameter, mm	0.6 (0.1)	0.6 (0.1)	0.97	0.5 (0.1)	0.3 (0.1)	0.08
Flow velocity, cm/s	7.4 (6.8)	8.0 (3.0)	0.9	6.5 (3.6)	8.8 (2.5)	0.3
BF, ml/s	0.5 (0.3)	0.6 (0.2)	0.40	0.4 (0.3)	0.3 (0.1)	0.5
Reactive Hyperemia						
Time to PV, s	45.8 (16.8)	12.0 (3.2)	0.004*	8.5 (3.2)	8.4 (1.5)	0.96
Peak TAMEAN, cm/s	26.8 (14.4)	50.9 (13.2)	0.007*	51.9 (3.26)	67.1 (30.5)	0.1
Peak TAMAX, cm/s	47.8 (26.9)	78.8 (19.9)	0.04*	89.0 (10.0)	105.0 (44.0)	0.2
Time to half RV, s	79.0 (27.7)	30.5 (14.6)	0.006*	25.3 (5.6)	20.0 (7.5)	0.2
Peak BF, ml/s	2.3 (1.8)	4.1 (1.7)	0.06	3.1 (1.4)	1.9 (0.6)	0.2
Peak SS AUC	12891(6333)	7328 (2017)	0.09	6803 (3442)	11225(6917)	0.1
Post-60s SS AUC	18707(11773)	27067(10303)	0.2	32258(13343)	51998(28628)	0.09

BF, blood flow; PV, peak velocity; TAMEAN, time-averaged mean velocity; TAMAX, time-averaged maximum velocity; RV. returned velocity; SS, shear stimulus; AUC, area under the curve. Values are means (SD).
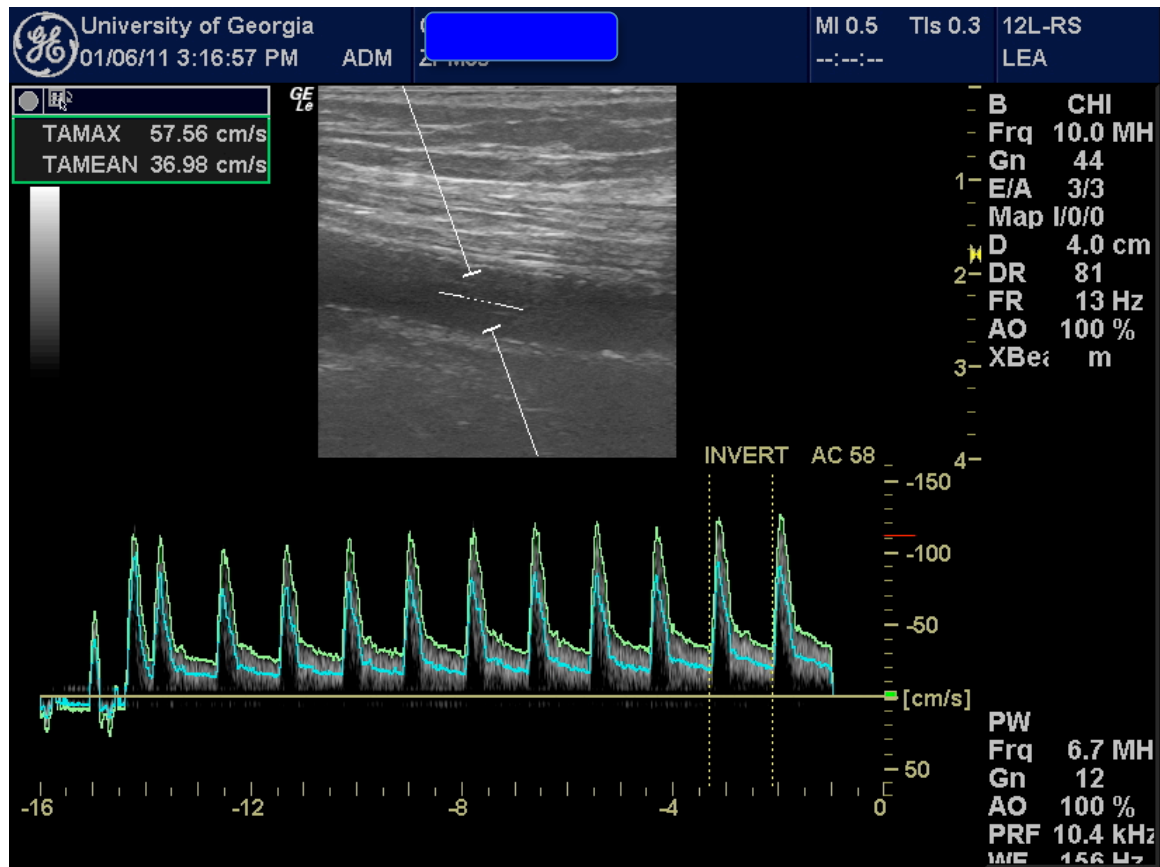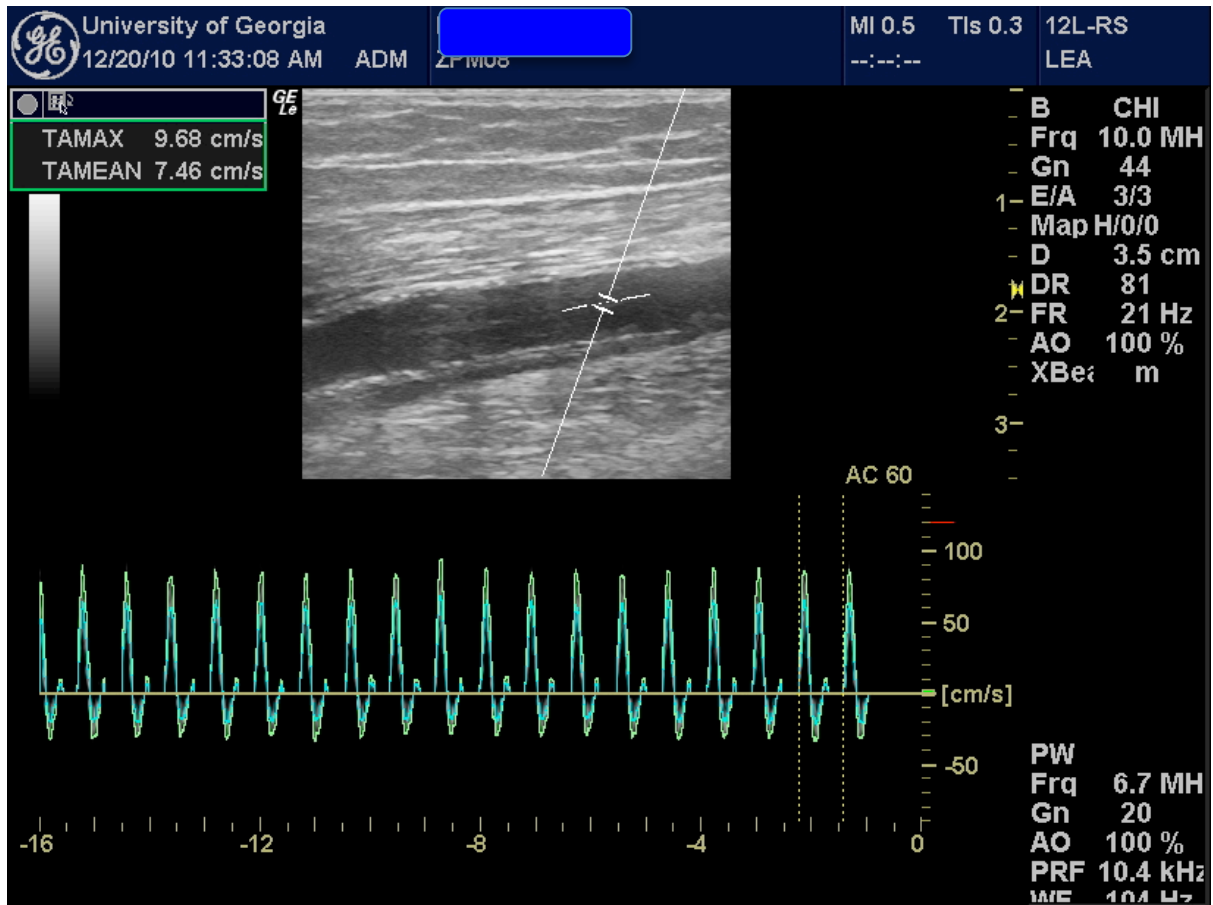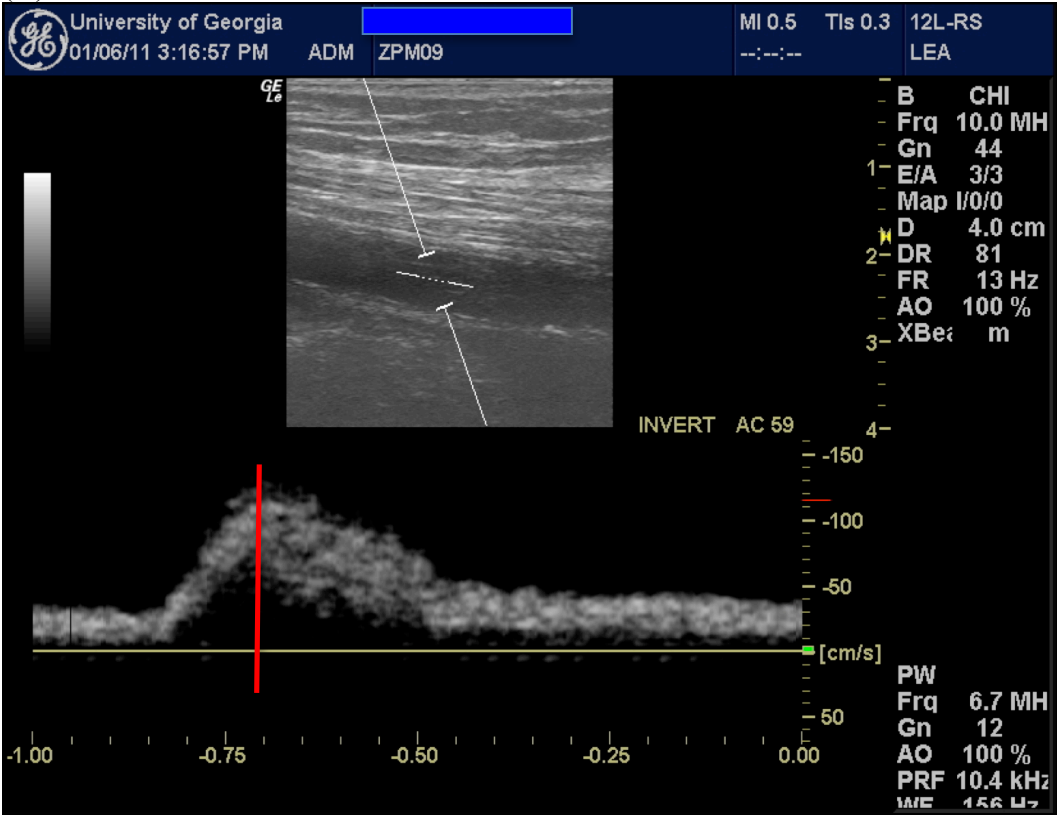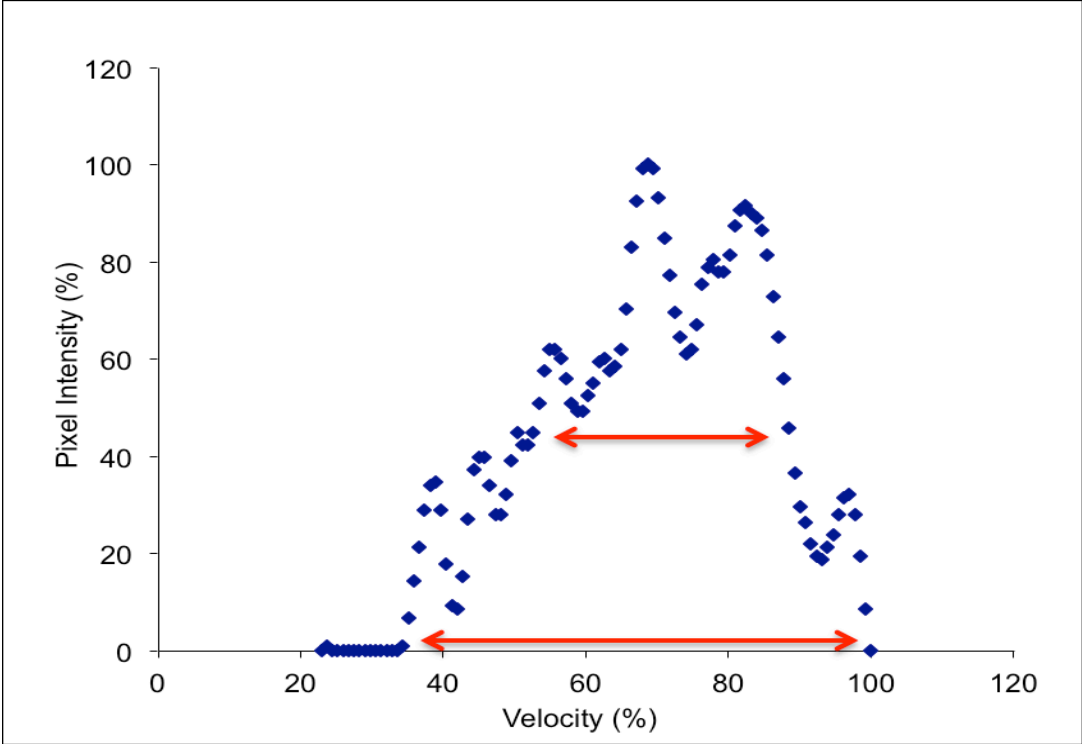
Figure 3.1

Figure 3.2

Figure 3.3

(A)

(B)

Figure 3.4

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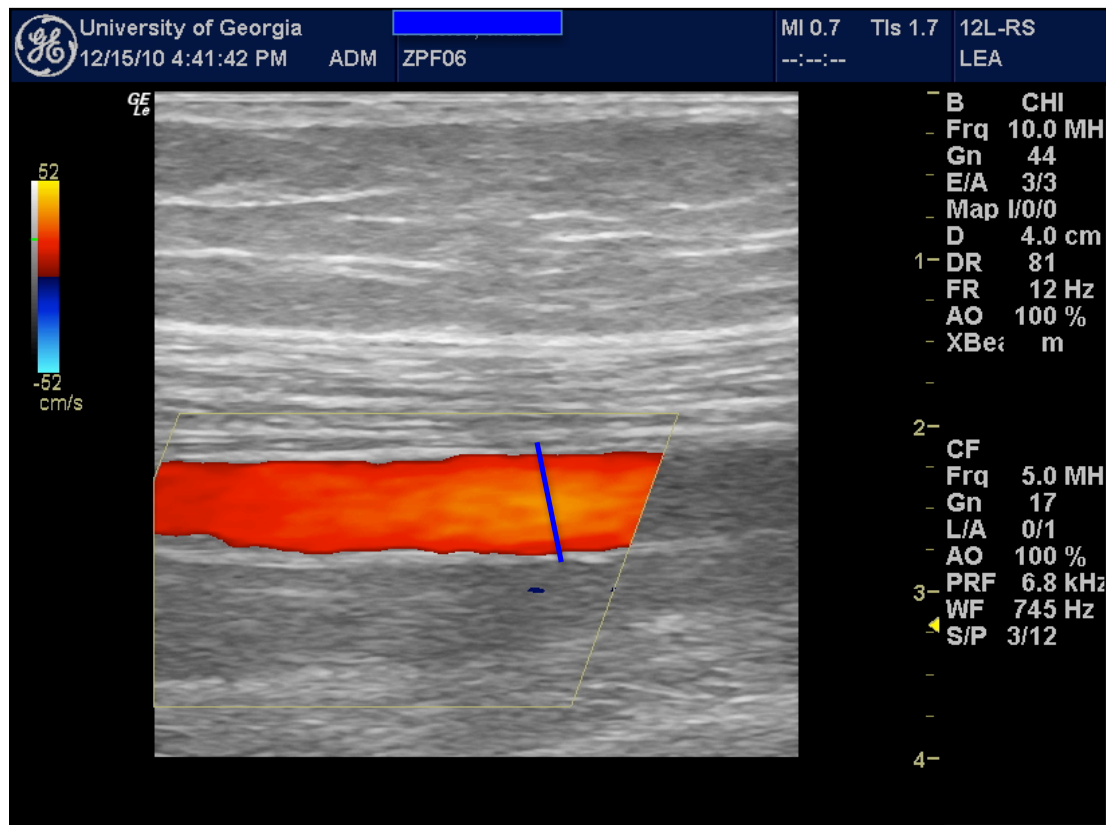
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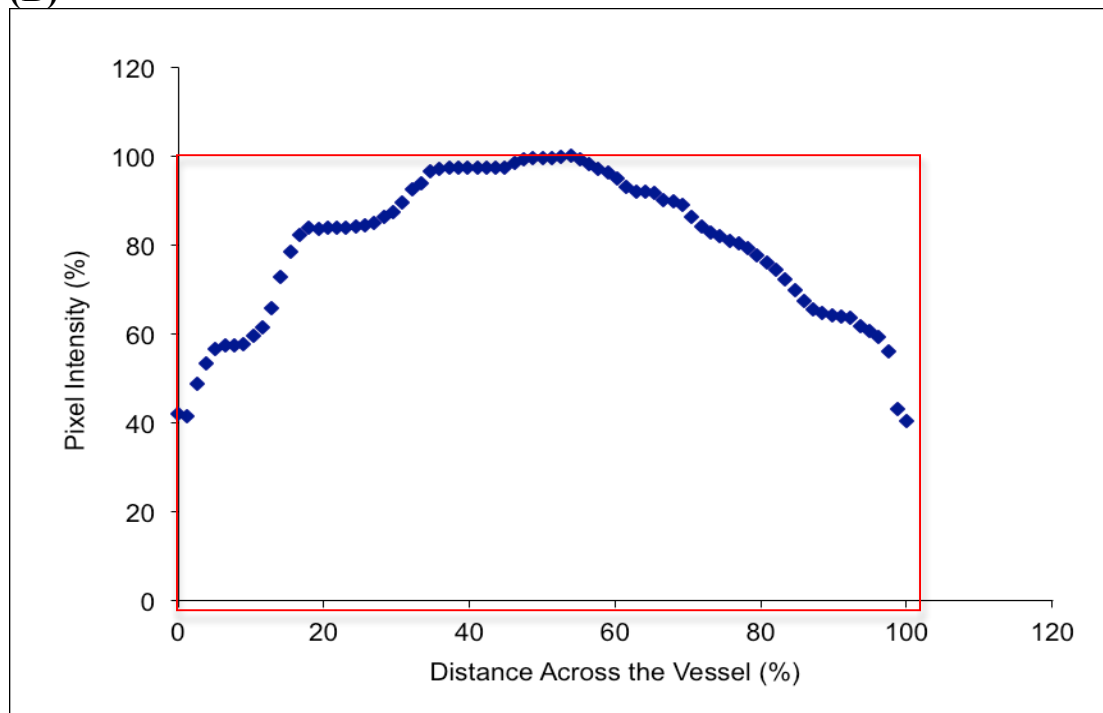
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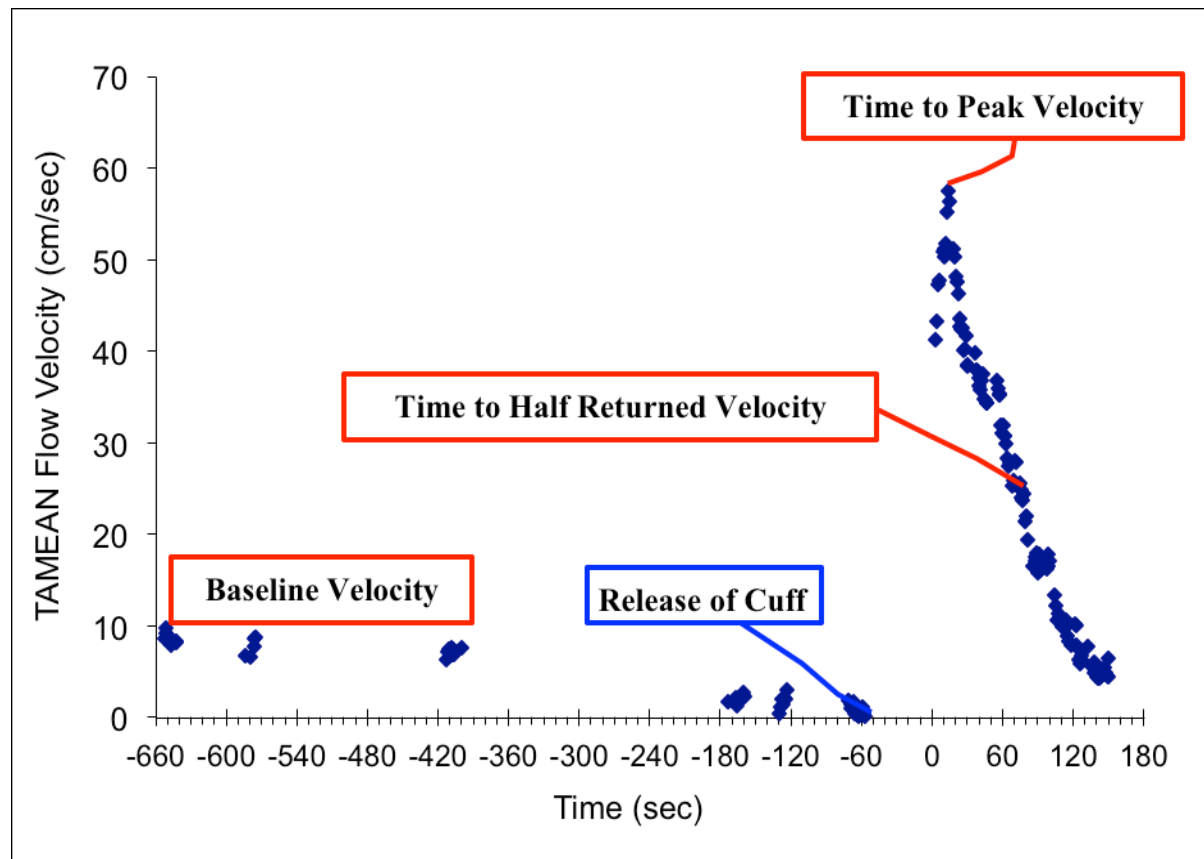
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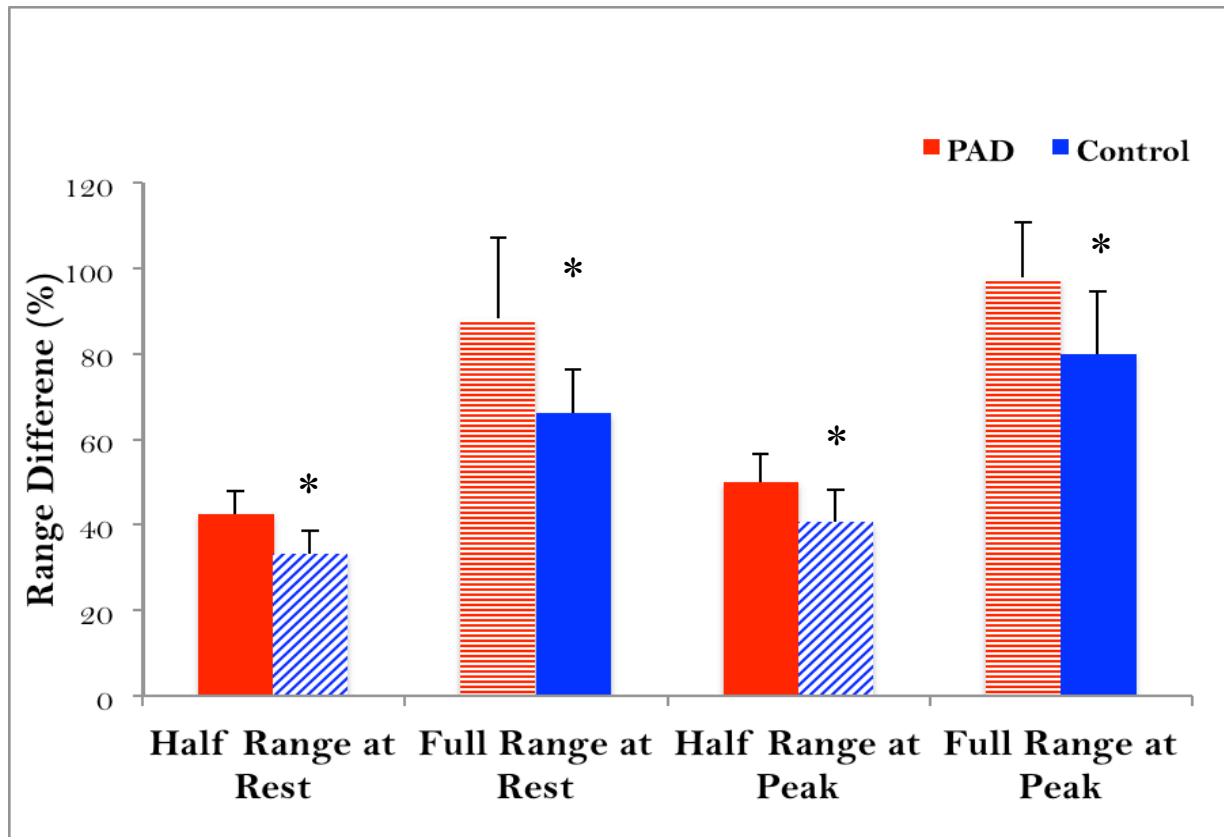
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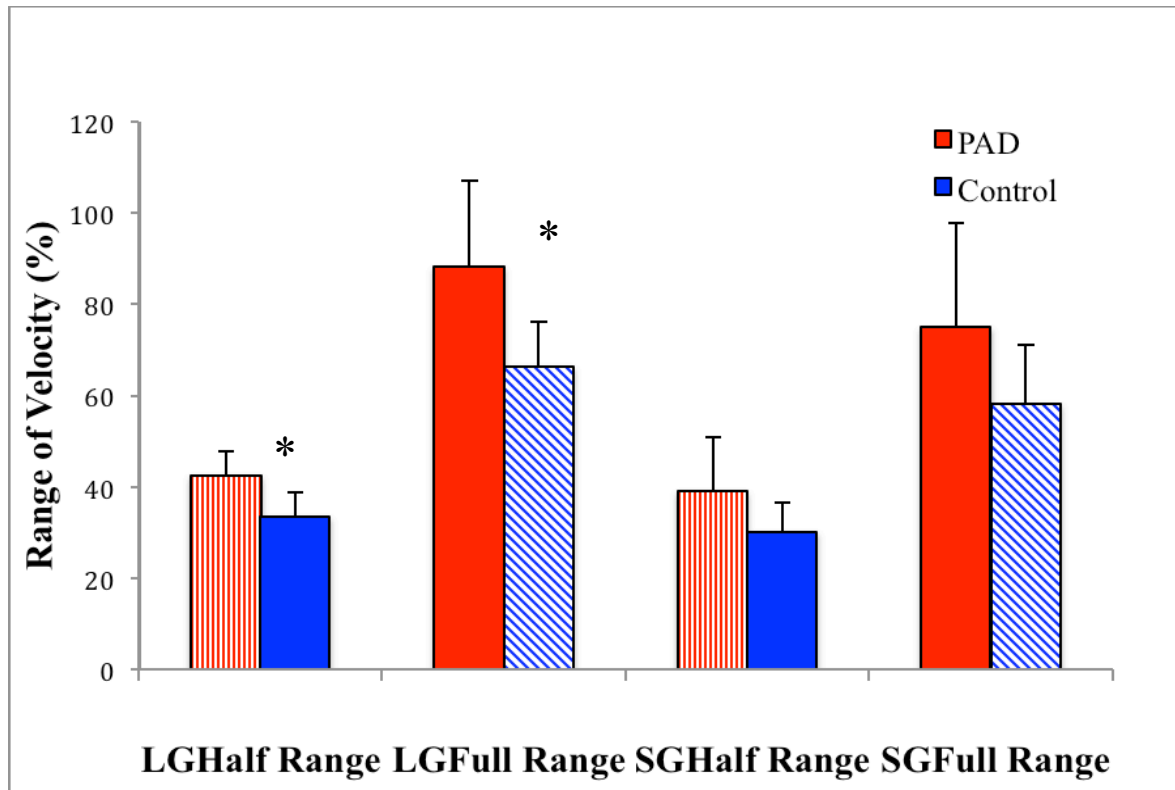
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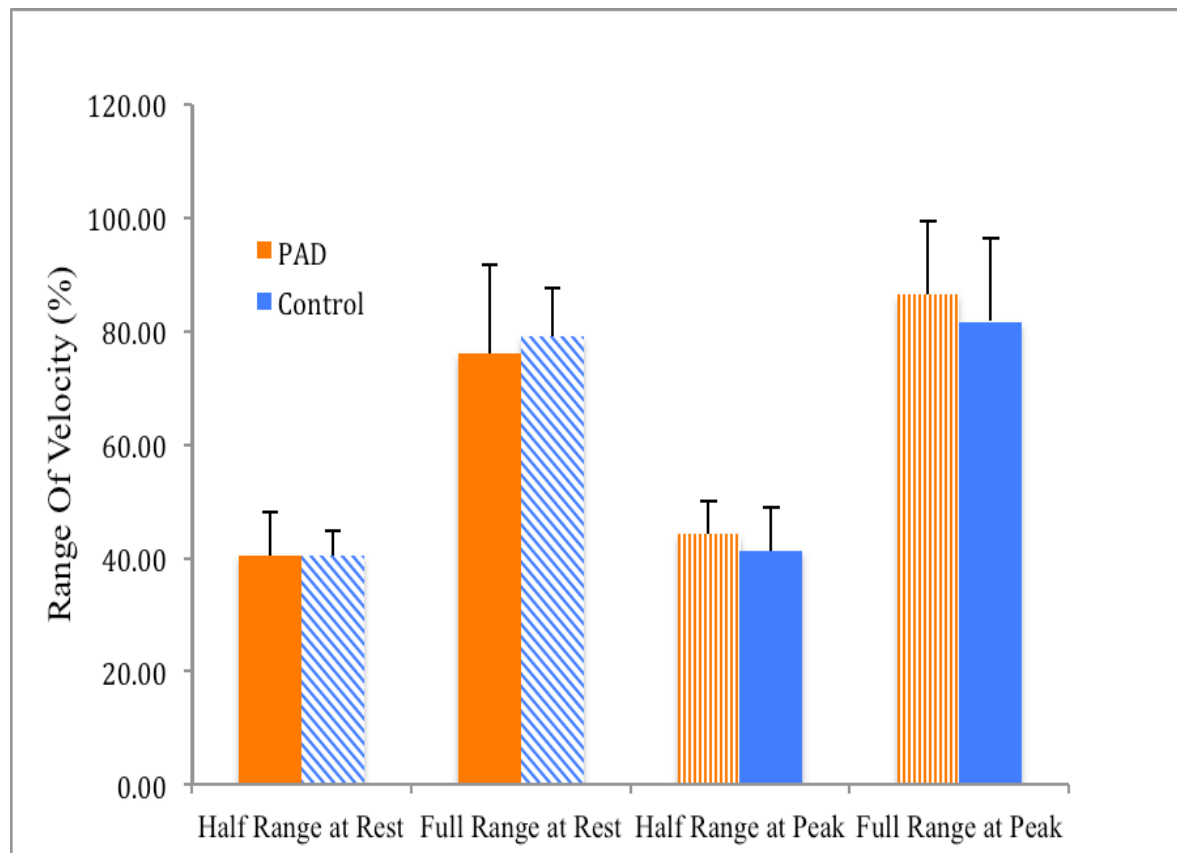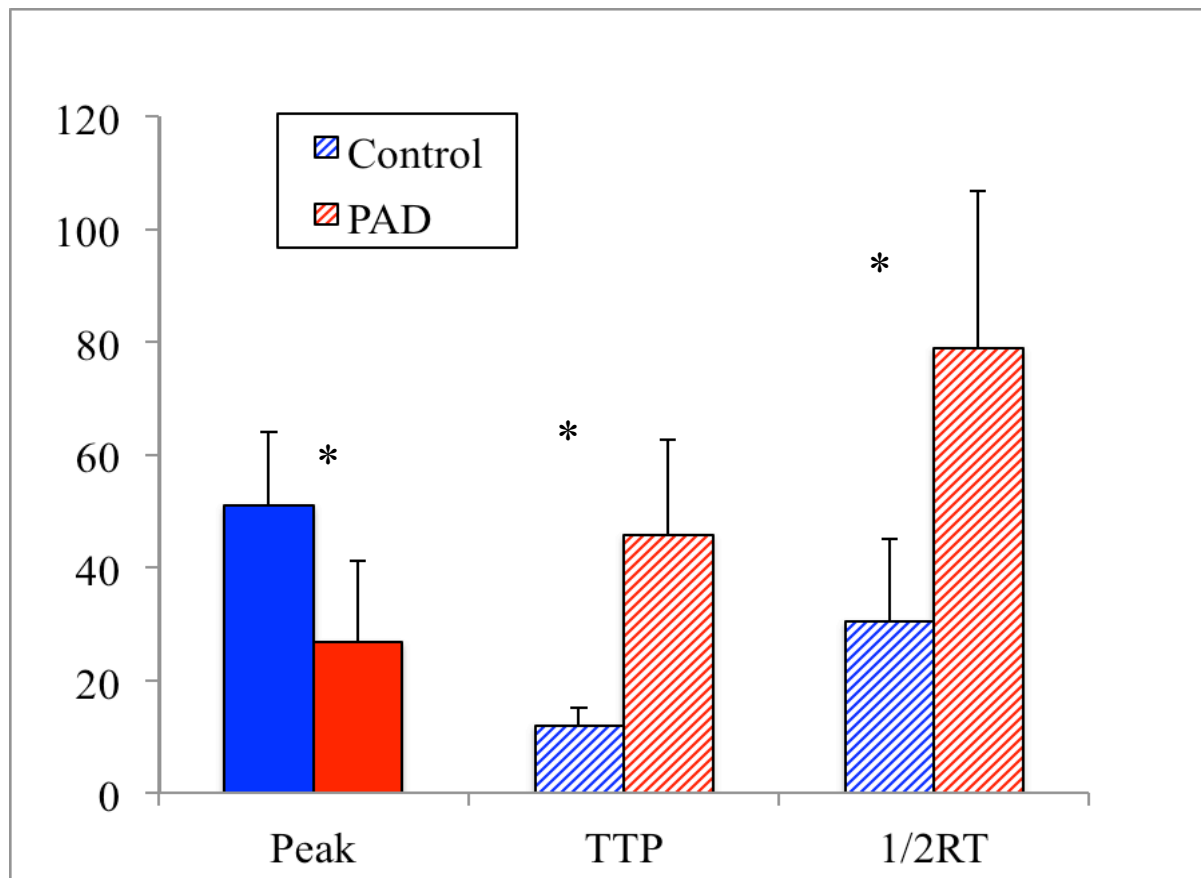
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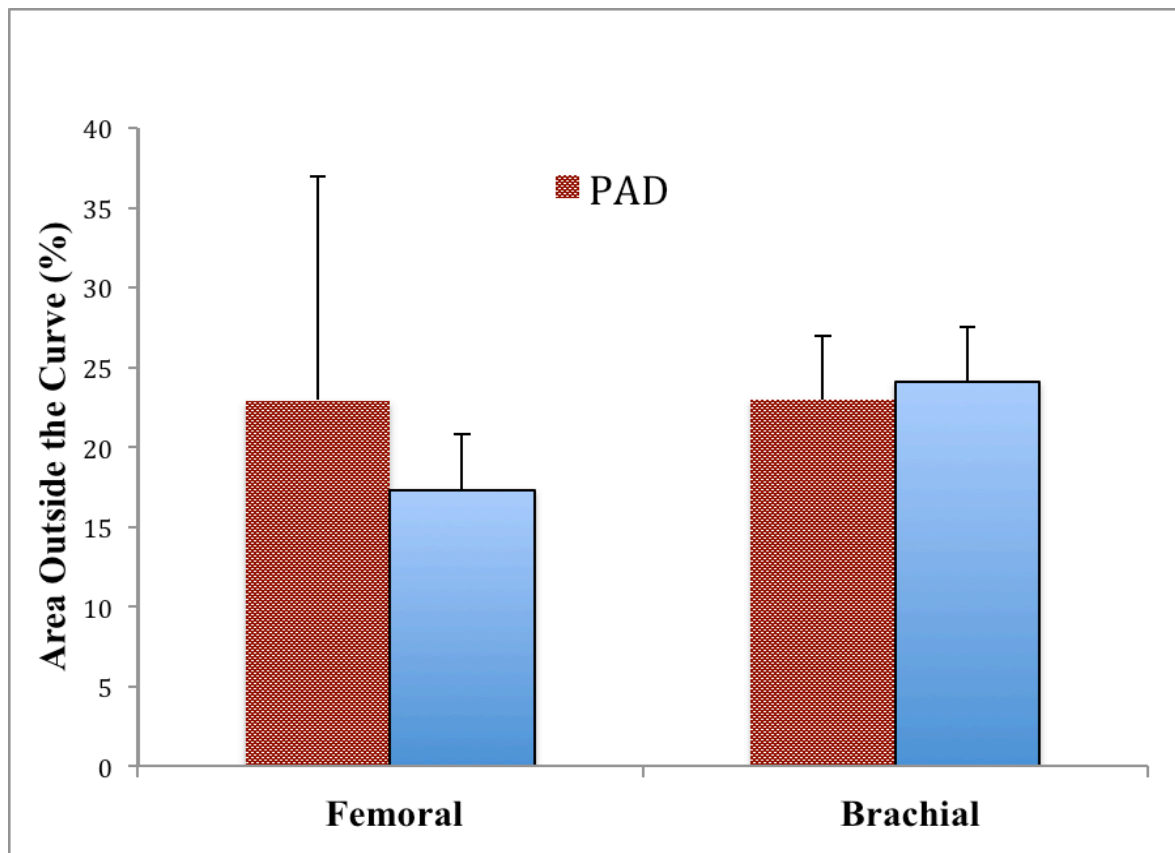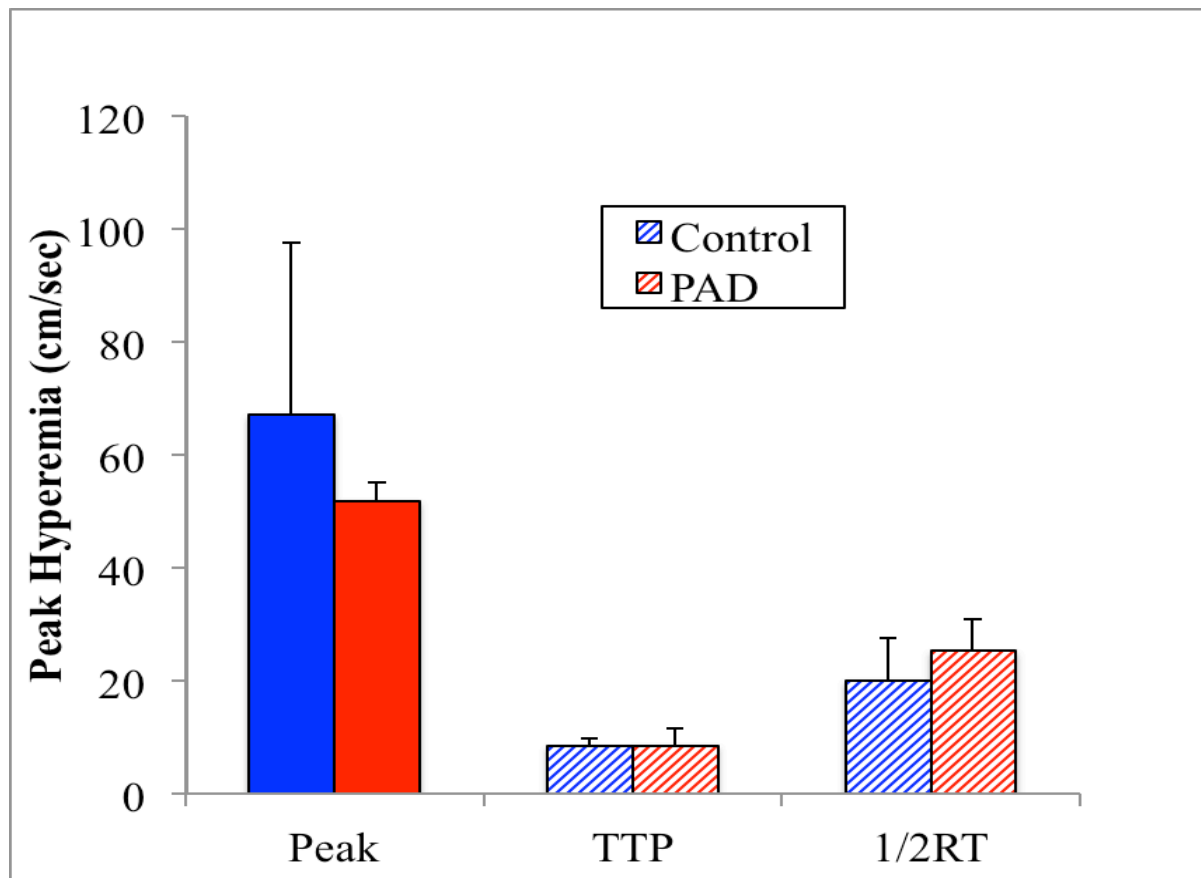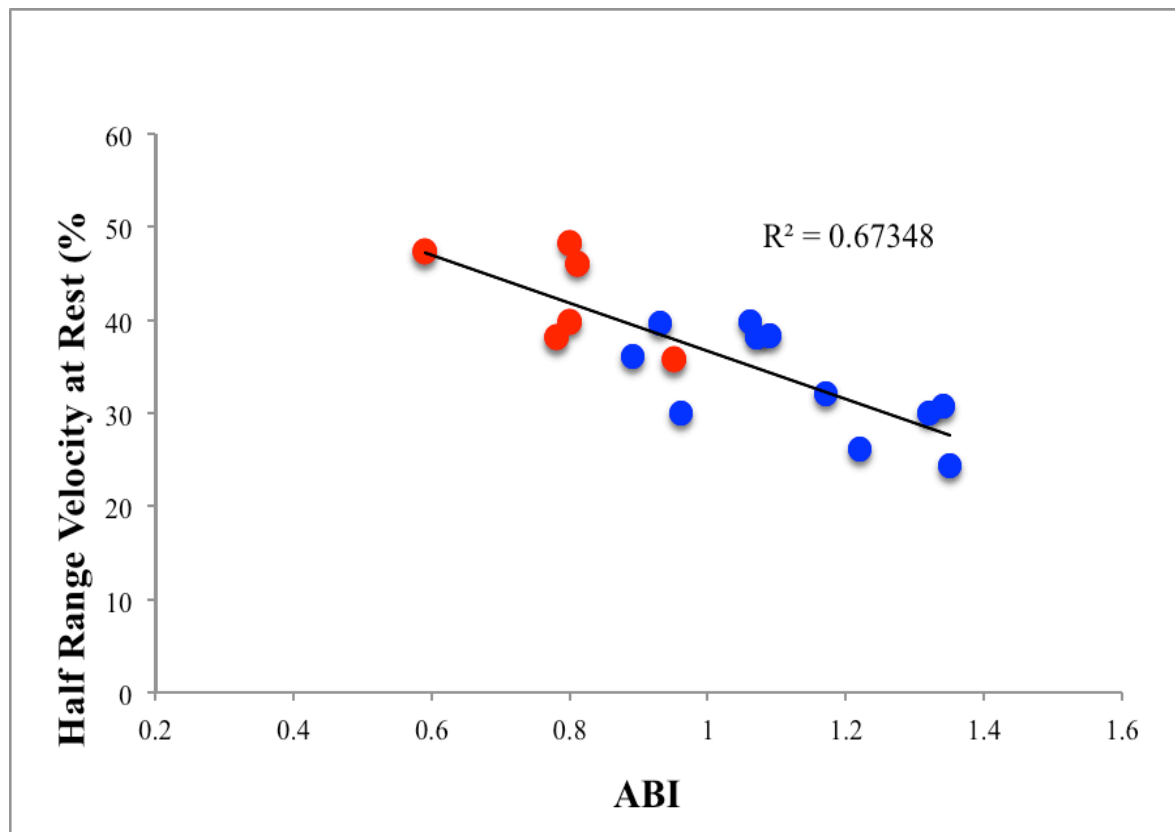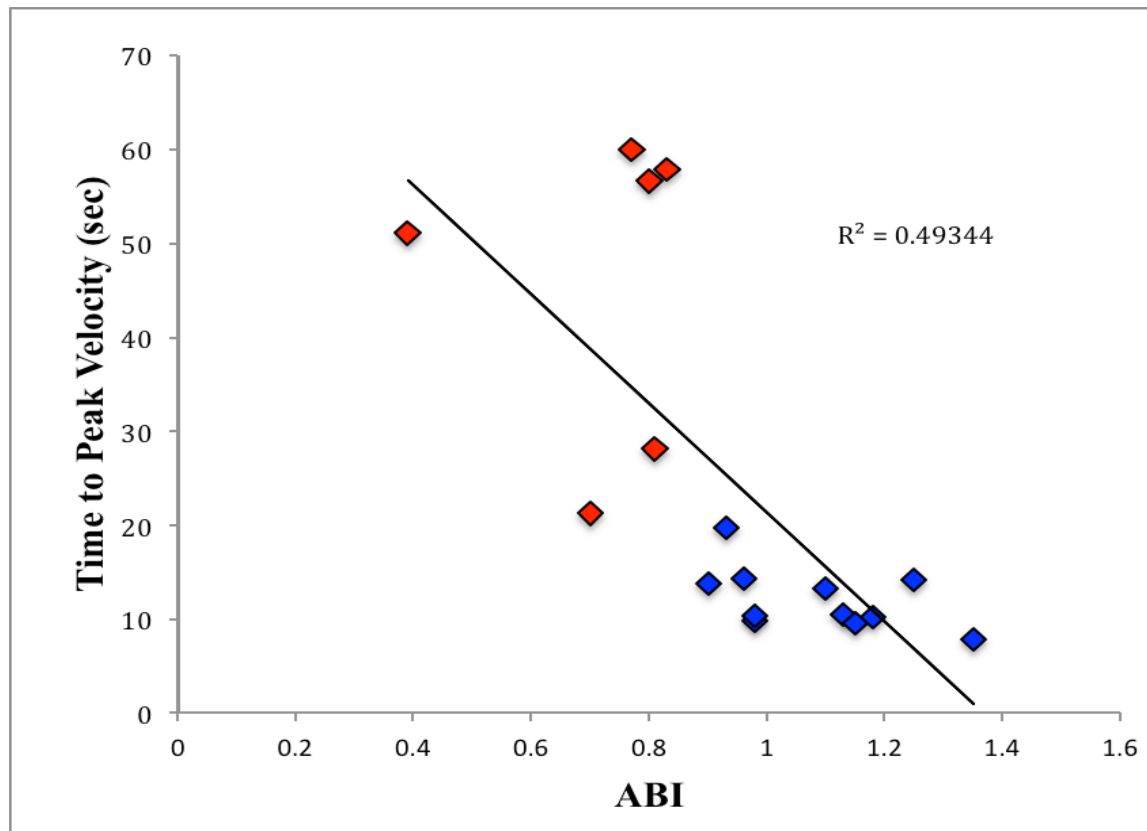
Figure 3.10

Figure 3.11

Figure 3.12

Figure 3.13

CHAPTER 4

SUMMARY AND CONCLUSION

Major Findings

One purpose of this study was to compare the velocity profile between the subjects with PAD and the subjects without. No significant difference was observed in flow velocity profile between groups, and this finding was consistent with the two measurements: spectral Doppler analysis and color image analysis. This finding was surprising, as we thought we would find significant differences in the velocity profile across arteries between groups, especially in the femoral artery. However, our results for the color images as well as the comparison of velocity range with the small gate to large gate are consistent and strongly support the conclusion that there are no meaningful differences in flow velocity profile between the subjects with and without PAD. We chose people with PAD as our target population because we felt that of all the possible disease groups, they were most likely to have differences in their velocity profile. If this finding is true, then it shows that future studies can assume a universal Posiuelle's law for all of their study participants, regardless of differences in cardiovascular health. Even as a negative finding, this result is important. Of course, futures studies will need to re-examine this effect to either support or confirm our findings.

After considerable deliberation with my advisor and colleagues, we thought that the half range and full range calculations were the best way to represent the broadness of Doppler spectra (variability of flow velocity). Perhaps the most difficult part of the spectral Doppler analysis was

to ensure that the analysis was done with the true pixel intensity (flow velocity) without including any artificial noise. Careful examination and adjustment of gain for each spectrum was performed to minimize this error. Acquiring good color flow images was technically difficult, and in perspective, it requires experimentation with color scales. Proper adjustment of color bar scale was necessary to avoid aliasing and to ensure optimal color contrast. It was noticed that color image could be easily modified by gain adjustment. Future investigation should be conducted to examine the change of velocity profile with gain adjustment.

Time course of reactive hyperemia was observed to be longer in people with PAD. This is consistent with a previous study performed by Shepherd in 1950 (22). Although his study mainly compared the magnitude of blood flow immediately after calf exercise, between subjects with and without intermittent claudication, there was evidence suggested that the time course of blood flow after exercise was longer in subjects with claudication. Interestingly, the magnitude of the delayed time course in the femoral artery found in this study was consistent with the severity of disease. However, we did not see differences in the time course when the brachial artery was compared between groups. This suggests that the delay in time course is a function of the ability to deliver oxygen to the tissues and is not necessarily an indicator of the health of blood vessels. Future studies will be needed to determine whether the delayed time course of reactive hyperemia is a useful and reliable indicator of disease severity.

Measuring Velocities Near Vessel Walls

One of the approaches used in this study to evaluate velocity across the vessel wall was to measure blood velocity using a small gate (1mm) in the center of the vessel and near the vessel wall. In practice, this approach did not seem to work well. We were able to get small-gate measurements in the center of the vessel but we were unable to obtain consist signals from the

small gate near the vessel wall. Most data obtained from this measurement was not reported in this study's analysis because some of the data had weak signal or shifts in gate position occurred. Additionally, the presence of plaque along arterial walls made it difficult to determine small gate placement. On the other hand, measuring flow velocity with gate across the vessel wall may be compromised by the nature of the narrow Doppler beams, as suggested by several studies, and this limitation could result in 33% of velocity overestimation (12, 41, 42). Despite the difficulty of using small gate to measure velocity near the walls, it might still be necessary to include this measurement in future studies. This will ensure the results remain consistent with what was found concerning both large and small gate at the lumen center in this study.

Measurement of Blood Viscosity

A potential limitation to our study was that blood viscosity was not measured. Posiuelle's law includes a term for blood viscosity and there was certainly a potential for differences in blood viscosity between our older subjects. Previous studies have suggested that the influence of individual variation on blood viscosity on shear stress is low. Padilla et al. (33) investigated the accuracy of shear stress by comparing shear stress calculated through both assumed blood viscosity and measured blood viscosity. They found that there was no difference between the two calculations and concluded that the addition of viscosity measurement might not have a significant effect on FMD results. However, it should be noted that most of the shear stress with blood viscosity studies were done on young, healthy individuals. In comparison, most of the participants in this study had at least one form of vascular disease. As reported by Leschke et al., there was an elevated blood viscosity in hypertensive patients when compared to the control group(24). Diabetes, obesity, and hyperlipidemia have also been found to be associated with increased blood viscosity (10, 25). It is possible that subjects with PAD have higher blood

viscosity; thus prolonging their time to peak velocity during reactive hyperemia. If this was true, however, we might have expected to see changes in time to peak velocity in the brachial artery, which we did not see. Future studies might consider measuring blood viscosity even if our study did not find evidence supporting its measurement.

NIRS Technology

Previous studies on people with PAD have used Near-infrared spectroscopy (NIRS) as a non-invasive method to measure the level of oxygen saturation in muscles. K. McCully et al. utilized NIRS to measure oxygen desaturation during exercise and suggested the usefulness of monitoring capacity of oxygen transport with NIRS in people with circulatory problems (26). Padilla et al. used NIRS to quantitatively demonstrate the forearm ischemia after cuff occlusion and concluded that it was necessary to take muscle ischemia into consideration because of its role in driving reactive hyperemia (33). The present study showed a longer time course of reactive hyperemia in the subjects with PAD. Due to experimental limitations, NIRS measurements were not made in our study. As mentioned earlier, prolonged time course of reactive hyperemia in the subjects with PAD might not necessarily be due to the compromised vascular health; as the time course was observed to be abnormal only in the femoral artery but not in the brachial artery. It will be interesting to see how the time course of reactive hyperemia is correlated with the magnitude of muscle ischemia when incorporating NIRS technology.

Study Participants & Necessity of Exercise Intervention

This study provided opportunities for me to meet people from diverse backgrounds. One of my conclusions was that my research participants were happy to help us in our experiments. The more they felt they were contributing to an important study, the more interested they were in helping. Telling the participants what they could do to help us while

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listening to them about what we could do to fulfill their needs is essential. The success of this study did not just require a good research plan and data collection, it involved developing a strong partnership between the researchers and study participants.

Almost all the participants showed the desire to be more physically active, especially the participants with PAD. Many of the participants with PAD understood the benefits of physical activities, but finding a suitable exercise program was challenging and prevented them from exercising for health. For instance, most of the existing exercise programs are targeted at individuals with higher physical capacity and therefore are not ideal for individuals with PAD. For people who are slow walkers, like most of the cases we found in people with PAD, participating in these exercise programs might be intimidating and might reduce the likelihood of being physically active. This suggests that there is a need to establish exercise-walking programs for individuals with PAD. Additionally, evaluating the time course of reactive hyperemia changes pre- and post- exercise-walking intervention may help us to further correlate characteristics of blood velocity to peripheral arterial disease severity.

CHAPTER 5

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