ACUTE EFFECTS OF EXERCISE & COMPRESSION THERAPY ON BLOOD FLOW AND FLOW-MEDIATED DILATION IN SMOKERS

by

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(Under the Direction of Kevin McCully)

ABSTRACT

Cardiovascular disease is the leading cause of death in the United States. Flow-mediated dilation (FMD) has been used as a non-invasive method of evaluating endothelial function and FMD is used as a marker for cardiovascular disease. It has been shown that the blood flow response during exercise is what improves FMD. Compression therapy has been hypothesized to have a blood flow response similar to that of exercise. Exercise has been shown to improve FMD and it is hypothesized that compression therapy will improve FMD.

The purpose of this study is to measure blood flow velocity during exercise and compression therapy and flow-mediated dilation (FMD) before and after a single bout of exercise and compression therapy in people who smoke.

Methods: Subjects were smokers age 22.5 +/- 2.56 years, smoked a pack of cigarettes or more a week, were relatively healthy, and not involved in any form of lower body training. There were 3 sessions where FMD was measured pre and post treatment (exercise, compression therapy, and time control) using a high-resolution ultrasound. Blood flow velocity was measured during treatment of exercise and compression therapy also using a high-resolution ultrasound.

Blood flow velocities and arterial diameters for FMD were collected and analyzed using advanced computer software.

Results: Exercise showed a higher total blood flow over time compared to baseline while compression therapy showed no difference in total blood flow when compared to baseline. Peak velocity averages were higher in exercise (~9 fold) and compression therapy (~3 fold) than baseline. There were no significant differences in FMD within or between treatments in this study.

Conclusion: Further research with different exercise method and higher pressures from compression device is needed to compare blood flow response and FMD changes with exercise and compression therapy.

INDEX WORDS: Flow-Mediated Dilation, Blood Flow Velocity, and Compression Therapy

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DEDICATION

To my mother

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Finally, I would like to thank my family. Without a strong foundation, no one would be able to get off the ground. Thank you all for being that for me and more.

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CHAPTER 1

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States and the world (13). Directly and indirectly, cardiovascular disease cost the United States billions of dollars yearly. Endothelial dysfunction may accelerate the arthrogenic process and serves as a marker of vascular health (59). Flow-mediated dilation (FMD) has been used as a non-invasive method of evaluating endothelial function (6), and FMD is an indicator for cardiovascular risk (3, 9, 47). Treatment of CVD risk factors has been shown to improve FMD (14), which include changing diet, refraining from smoking, and physical activity. Exercise has been shown to improve FMD (4, 17, 31, 38, 39, 43). The time frame in which FMD improves with exercise is not yet certain. It has been shown to improve after one week (1), but it is not clear if less than one week is sufficient to improve FMD.

Certain special populations such as people with spinal cord injury (SCI) have impaired FMD (50, 53). Spinal cord injuries result in inactivity of affected region of the body. Lower extremities tend to be most often affected because of anatomical locations of nuclei of the spinal cord. A decrease in blood flow and decreased flow-mediated dilation is observed in SCI patients (50, 53). Because of inability to exercise train in the traditional methods, novel methods have to be used by SCI patients such as electrical stimulation (20, 50, 53, 54). Electrical stimulation can be difficult for SCI patients due to the fact that they can have little to no use of their arms, mobility issues, and balance and coordination difficulties.

Exercise improves FMD and therefore endothelial function, but it has been suggested that improvements are related to blood flow response due to exercise (27). The rationale behind this is that exercise exerts repetitive increases in shear stress on the endothelium. Through these increases in shear stress the endothelium's function and structure is improved and therefore lowering atherosclerotic risk.

With the rationale of some populations not being able to traditionally exercise and exercise improves cardiovascular health through blood flow increases, non-traditional forms of exercise that increases blood flow need to be considered. Electrical stimulation has been studied (20, 50, 53, 54). Other unique treatments have also been studied. External counter-pulsation (ECP) therapy has been shown to improve FMD (48), but SCI patients would find ECP therapy difficult because of the high pressures used (300 mmHg) and rapid inflation times (~1/3s) of the garments are too dangerous for someone who does not have sensory feedback from their lower extremities and this could lead to skin lesions. The use of intermittent pneumatic foot compression has been used and hemodynamic characteristics in the lower limb post treatment of compression has been measured (7, 12). Positive repeatability and increases in flow were measured in these studies as well using lower limb compression. The use of venous compression therapy is similar to that of ECP, except the therapy uses lower pressures and slower inflation time which eliminates the risks seen with ECP to the lower extremities. Little is known about compression therapy and its use outside of the clinical use to treat deep vein thrombosis, varicose veins, edema, leg ulcers, and chronic venous insufficiency (33, 44). Since there are many similarities in the compression therapy and that of external counter-pulsation therapy, it is hypothesized that FMD will improve with compression therapy.

Smokers have been shown to have impaired FMD (11, 19, 21, 24, 32, 42, 45, 49, 55, 58). Knowing this, smokers are an at-risk population with the possibility of improving FMD with exercise or the use of a non-traditional method like that of compression therapy.

Statement of the Problem

Venous compression therapy has been marketed as a method to improve arterial health, but is not clear what the magnitude of flow increase is with this treatment. In addition, it is not clear how many bouts of increased blood flow are needed to enhance arterial health. The problems of the study are to determine a single bout of moderate exercise will affect flow-mediated dilation and determine if compression therapy will cause a blood flow response similar to that of moderate exercise in smokers. Finally, the study will determine if a single bout of compression therapy will affect FMD.

Specific Aims

1) To measure and compare the blood flow response in smokers during exercise and compression therapy.

2) To measure flow-mediated dilation before and after a single bout of exercise and a single bout of compression therapy to see if FMD improves.

Hypotheses

It is hypothesized that:

1) Compression therapy will increase blood flow similar to that of moderate exercise
2) A single bout of exercise/compression therapy will improve FMD in smokers

Significance of the Study

Exercise has numerous health benefits. There have been suggestions that one of the benefits of exercise is related to the increase shear stress produced by exercise (27). Because some people are either unable to exercise or have difficulty in exercising, the use of alternatives to exercise that produce the same increases in blood flow might be of value. There is an ongoing interest in SCI patients and the possibility of improving the daily life and health of that population with methods other than traditional exercise. This is particularly true with the prevention of cardiovascular disease through improvements in endothelial function. So while it is known that non-traditional exercise training, like electrical stimulation, improves FMD in SCI populations (50), there is little known about if the blood flow response in exercise is responsible for the improvements in endothelial function. If that blood flow response is enough to improve endothelial function then a non-traditional method that increases blood flow for an extended time could improve FMD and therefore improve endothelial function.

CHAPTER 2

Review of Related Literature

Cardiovascular Disease and Flow-Mediated Dilation

Flow-mediated dilation has been reported as an independent predictor of cardiovascular risk. A study aimed at assessing whether brachial artery FMD along with ankle-brachial pressure index was an indicator of cardiovascular risk. One hundred thirty-one peripheral arterial disease patients were monitored for a mean of 23+/-10 months. Median FMD was lower in patients that had a cardiovascular event than those without events (5.8% versus 7.6%, $p<0.05$). When grouped by FMD, above or below median FMD (6.7%), there was a higher number of cardiovascular events in the below median FMD than in the above median FMD (3). Similarly, Shechter et al. (47) monitored 435 middle-aged (54+/-12 years) healthy subjects for 32+/-2 months. Subjects were divided into an above and below median FMD (10.7%). Coronary artery disease risk factors were comparable in both groups. After 32+/-2 months there were significantly more cardiovascular events in subjects with below median FMD than in subjects with above median FMD (11.8% versus 4.7%, $p=0.007$).

Measuring Flow-Mediated Dilation

Traditionally FMD is measured in the brachial artery (5). Baseline diameters are acquired then an occlusion cuff is placed and inflated distally to where the ultrasound probe is placed and readings are acquired. The cuff stays inflated at a pressure of 100mmHg over systolic blood

pressure for a duration of 5 minutes. Diameters are recorded after the release of the cuff to acquire a peak diameter of the vessel due to the reactive hyperemic response from the release of the cuff. Flow-mediated dilation is calculated as a percentage of the difference between peak diameter and resting diameter divided by resting diameter (50). Recent studies have extended this research to other arteries, including the legs (29). Recent studies have also shown the importance of controlling for initial diameter and the magnitude of the shear stress stimulus (49).

Impaired Flow-Mediated Dilation in Smokers

Flow-mediated dilation has been reported to be reduced in smokers (11, 19, 21, 24, 32, 42, 45, 49, 55, 58). Most studies report a decrease in FMD of approximately 50% in people who smoke or use tobacco. For example, in one study (55), 616 healthy Chinese (23% were smokers) had FMD measured and the Chinese smokers had significantly lower FMD when compared to nonsmokers (7.0+/-2.3 versus 8.2+/-2.5%, $p<0.001$).

In a second study (11) where 20 healthy long-term smokers (15 males, 5 females, mean age 27+/-9 years, smoking average of 25 cigarettes/day, and smoking average of 10+/-2 years) and 20 healthy nonsmoking hospital staff (14 males, 6 females, mean age 25+/-7 years) had their FMD measured and compared. FMD was significantly lower in smokers when compared to non-smokers (4.7+/-1.6% versus 9.2+/-4.6%, $p=0.0001$).

Stoner et al. performed two studies on occasional smokers. In the first study (49) they reported that occasional smoking is associated with a general vasoconstriction, which can interfere with the interpretation of the FMD results. In the second study (49), Stoner et al occasional smokers have evidence of impaired function two days after they had stopped smoking, and that smoking two cigarettes resulted in a further decrease in FMD. Young

nonsmokers (age 20-26 years, n=9) and occasional smokers (<1 pack/week, n=9) were measured. Occasional smokers showed shear rate-diameter slope was reduced by 35.9% compared with nonsmoking controls. These studies showed that occasional smokers also have impaired arterial function (49), and that acute cigarette smoking causes vasoconstriction that needs to be accounted for when calculating FMD.

Exercise and Improving Flow-Mediated Dilation

A number of studies have shown that impaired FMD can be improved with exercise. A 4-month study by Fuchsjager-Mayrl et al. (17) where 26 participants with type 1 diabetes (20+/-10 years duration) were grouped into bicycle exercise training program (n=18, age 42+/-10 years) and non-training controls (n=8, age 33+/-11 years). Training duration was ~ 1 hour starting with warm-up and reaching a heart rate of 60-70% of heart rate reserve that lasted over 40 minutes then a 5 minute cool-down. Training was 2 times/week for first 2 weeks, then for the remainder of the program, training was 3 times/week. Exercise training lasted a total of 4 months. FMD was measured baseline, 2 and 4 months of training, and 8 months after training had stopped. There were significant improvements in FMD after training (from 6.5+/-1.1 to 9.8+/-1.1%, p=0.04) when compared to the control group where there was no significant difference in FMD.

Allen et al. (1) also showed that FMD could improve with exercise. Fourteen healthy males (age 26+/-5.7 years) had FMD measured before (V1), during (V2-V7), and at the end of training (V8). Training lasted 4 weeks and consisted of 60% of max voluntary handgrip contraction (20 min, 1/day, 5 days/week). There was an increase in FMD from V1 to V8 of

62%. Also interesting, was that there was significant FMD improvements between V1 and V2, where V2 was only after 4 training days.

Exercise training can improve FMD in SCI patients as seen in a study by Stoner et al. (50). Five 36+/-5 year-old SCI males had FMD measured before, during, and after training of quadriceps femoris muscle group electrical stimulation of both legs. Training was 18 weeks long (4 x 10 repetitions of unilateral, twice/week, dynamic knee extensions). Flow-mediated dilation was measured in the posterior tibial artery. FMD was improved from 0.08+/-.11mm (2.7%) to 0.18+/-.15mm (6.6%) ($p=0.004$) after 18 weeks.

Oftentimes, when looking at vascular adaptations and assessment, hyperemic response or total blood flow is considered. There have been debates about the stimulus for vascular adaptations (26). It is understood that the stimulus to improve endothelial health is shear stress (8), but does that shear stress have to be total blood flow increase related? Endothelium-dependent vascular dilatation could be due to increases in local blood flow in the active limbs or be a product of increases in accelerated local flow that signals endothelial cells through shear stress (46). Green et al. looked at lower limb exercise, cycling, and observed changes in blood flow in the brachial artery (40). Changes in mean flow in the brachial artery agreed with that of the prediction of redistribution of blood flow. So total flow did not increase but the magnitude of anterograde and retrograde did increase, changing the pattern of blood flow. The oscillations in flow, where the blood is dragged during anterograde and retrograde across the endothelial wall, is assumed to be a great enough stimulus for endothelial nitric oxide (NO) production and bioavailability (26, 27). Green et al. found that NO blockade during cycling was only slightly higher than during handgrip exercise (25). This shows that with similar total flow, mode and intensity of exercise are important to consider for pattern of blood flow.

Importance of Blood Flow

Exercise is important to health in particular is important to vascular health. Research has shown that exercise improves FMD in humans (1, 17, 50). Exercise improves FMD, but what aspect of exercise specifically improves FMD? Exercise involves neural responses, metabolic exchanges, circulatory responses, and more. Green et al. (27) suggests that the blood flow response seen during exercise is what is important to vascular health. Exercise can help maintain a healthy weight, lower cholesterol, and improve blood pressure in humans. These are all improvements that can decrease cardiovascular risk, but Green et al. (27) reports that shear stress on the endothelium seen during exercise could account for the positive effects beyond that of traditional risk factor improvements.

Compression Therapy

One of the non-traditional methods that have been considered is arterial compression, called external counter-pulsation (ECP) therapy. ECP therapy has been shown to improve FMD (48). Twenty CAD patients (15 males and 5 females, average age 68+/-11 years) had FMD measured before and after ECP treatment. Shechter et al. compared the ECP treatment group with a control group with similar age and same number of males and females. External counter-pulsation treatment group received 5 1-hour sessions/week for 7 weeks for a total of 35 hours of ECP treatment. Post-intervention FMD was improved in the ECP treatment group (8.2+/-2.1%, $p=0.01$) compared to that of controls (3.1+/-2.2%, $p=0.78$). The limitation to using ECP is that very high pressures gradients are used (300 mmHg in less than a second), and the equipment is costly (~$80,000). The potential for skin lesions or autonomic dysregulation is significant, and thus ECP may not be appropriate for many exercise intolerant populations, particularly people with SCI.

The use of venous compression therapy is similar to that of ECP, except the therapy uses lower pressures (~100 mmHg) and slower inflation time (30 seconds) which eliminates the risks seen with ECP to the lower extremities. Little is known about compression therapy and its use outside of the clinical use to treat deep vein thrombosis, varicose veins, edema, leg ulcers, and chronic venous insufficiency (33, 44). In particular, there are no published data on what kind of increases in arterial blood flow can be expected, and whether these increases in flow can influence arterial health. Since there are many similarities in the compression therapy and that of external counter-pulsation therapy, it is hypothesized that FMD will improve with compression therapy.

The aims of the study are to measure and compare the blood flow response in smokers during exercise and compression therapy and to measure flow-mediated dilation before and after a single bout of exercise and a single bout of compression therapy to see if FMD improves. It is hypothesized that compression therapy will increase blood flow similar to that of moderate exercise and a single bout of exercise/compression therapy will improve FMD in smokers.

CHAPTER 3

ACUTE EFFECTS OF EXERCISE AND COMPRESSION THERAPY ON BLOOD FLOW AND FLOW-MEDIATED DILATION IN SMOKERS

T T Nguyen, K McCully, L Nielsen, and K Shipp to be submitted to the Journal of Applied Physiology

Abstract

Cardiovascular disease is the leading cause of death in the United States. Flow-mediated dilation (FMD) has been used as a non-invasive method of evaluating endothelial function and FMD is used as a marker for cardiovascular disease. It has been shown that the blood flow response during exercise is what improves FMD. Compression therapy has been hypothesized to have a blood flow response similar to that of exercise. Exercise has been shown to improve FMD and it is hypothesized that compression therapy will improve FMD.

The purpose of this study is to measure blood flow velocity during exercise and compression therapy and flow-mediated dilation (FMD) before and after a single bout of exercise and compression therapy in people who smoke.

Methods: Subjects were smokers age 22.5 +/- 2.56 years, smoked a pack of cigarettes or more a week, were relatively healthy, and not involved in any form of lower body training. There were 3 sessions where FMD was measured pre and post treatment (exercise, compression therapy, and time control) using a high-resolution ultrasound. Blood flow velocity was measured during treatment of exercise and compression therapy also using a high-resolution ultrasound. Blood flow velocities and arterial diameters for FMD were collected and analyzed using advanced computer software.

Results: Exercise showed a higher total blood flow over time compared to baseline while compression therapy showed no difference in total blood flow when compared to baseline. Peak velocity averages were higher in exercise (~9 fold) and compression therapy (~3 fold) than baseline. There were no significant differences in FMD within or between treatments in this study.

Conclusion: Further research with different exercise method and higher pressures from compression device is needed to compare blood flow response and FMD changes with exercise and compression therapy.

Introduction

It is widely accepted that exercise has beneficial effects on health for human subjects(15, 34, 39, 51, 52, 56, 57). Benefits range from bone structure improvements to lowering impairments seen in multiple sclerosis patients to lowering cardiovascular disease risk factors. Cardiovascular disease (CVD) is the leading cause of death in the United States and the world (13). Directly and indirectly, cardiovascular disease cost the US billions of dollars yearly. Endothelial dysfunction may accelerate the arthrogenic process and serves as a marker of cardiovascular health (59). Flow-mediated dilation (FMD) has been used as a non-invasive method of evaluating endothelial function (6), and FMD is an indicator for cardiovascular risk (3, 9, 47). Treatment of CVD risk factors has been shown to improve FMD (14), which include changing diet, refraining from smoking, and physical activity. Exercise has been shown to improve FMD (4, 17, 31, 38, 39, 43). The time frame in which FMD improves with exercise is not yet certain. It has been shown to improve after one week (1), but it is not clear if less than one week is sufficient to improve FMD.

There have been debates about the stimulus from exercise that leads to vascular adaptations (26). It is understood that the stimulus to improve endothelial health is shear stress (8), but does that shear stress have to be total blood flow increase related? Endothelium-dependent vascular dilatation could be due to increases in local blood flow in the active limbs during exercise or be a product of increases in accelerated local flow that signals endothelial

cells through shear stress (46) that could be caused by exercise in a different region of the body or a pseudo-exercise treatment. It has been shown that lower limb exercise, cycling, can show changes in blood flow in the brachial artery (40). In this study, total flow did not increase but the magnitude of anterograde and retrograde did increase, changing the pattern of blood flow. The oscillation in flow, where the blood is dragged during anterograde and retrograde across the endothelial wall, is assumed to be a great enough stimulus for endothelial nitric oxide production and bioavailability (26, 27). It has also been shown that NO blockade during cycling is only slightly higher than during handgrip exercise (25). This shows that with similar total flow, mode and intensity are important to consider for pattern of blood flow when referring to exercise and its affect on blood flow pattern.

Certain special populations such as people with spinal cord injury (SCI) have impaired FMD (50, 53). Spinal cord injuries result in inactivity of affected region of the body. Lower extremities tend to be most often affected because of anatomical locations of nuclei of the spinal cord. Exercise is difficult for this population and other clinical populations. A decrease in blood flow and decreased flow-mediated dilation is observed in SCI patients (50, 53). Because of inability to exercise train in the traditional methods, novel methods have to be used to increase shear stimulus in such populations such as electrical stimulation (20, 50, 53, 54). Electrical stimulation can be difficult for SCI patients due to the fact that they can have little to no use of their arms, mobility issues, and balance and coordination difficulties.

With the rationale of some populations not being able to traditionally exercise and exercise improves cardiovascular health through blood flow increases, non-traditional forms of exercise that increases blood flow, shear stimulus, need to be considered. There have been non-traditional methods that have been considered. External counter-pulsation (ECP) therapy has

been shown to improve FMD (48), but SCI patients would find ECP therapy difficult because of the high pressures (300mmHG) used and rapid inflation times (~1/3s) of the garments are too dangerous for someone who does not have sensory feedback from their lower extremities which could lead to skin lesions. The use of venous compression therapy is similar to that of ECP, except the therapy uses lower pressures and slower inflation time which eliminates the risks seen with ECP to the lower extremities. Little is known about compression therapy and its use outside of the clinical use to treat deep vein thrombosis, varicose veins, edema, leg ulcers, and chronic venous insufficiency (33, 44). Since there are many similarities in the compression therapy and that of external counter-pulsation therapy, it is hypothesized that FMD will improve with compression therapy.

The purpose of the study was to observe shear stimulus of compression therapy to that of exercise and measure FMD before and after a single treatment of compression therapy and exercise in an impaired population. Smokers have been shown to have impaired FMD (11, 19, 21, 24, 32, 42, 45, 49, 55, 58). Knowing this, smokers are an at-risk population with the possibility of improving FMD with exercise or the use of a non-traditional method like that of compression therapy.

Methods

Experimental Design

This study is a pre-post, interventional design study. There were a total of three 1-hour sessions that were either an exercise, compression therapy, or a time control session. All testing and training was conducted in the Vascular Biology lab in the Department of Kinesiology at the University of Georgia.

Participants

A total of 10 men and 1 woman were recruited in the Athens area for this study. Subjects were average age (22.4±2.16 years), relatively healthy with the exception of smoking, regular smokers, and performing less than once per week of lower body exercise training within the last 6 months. The study was approved by the Institutional Review Board (IRB) and study participants all read and signed an informed consent statement.

Sessions

There were three sessions that subjects were required to attend. Each session was approximately 2-hours long. Each session consisted of a test of flow-mediated dilation prior to and after the treatment which were exercise, compression therapy, and a time control. Prior to measuring pre and post FMD, subjects were rested in the supine position for 10 minutes to stabilize hemodynamics in the subjects. During the sessions, blood flow velocities were also captured to quantify the blood flow stimulus of each treatment and to allow for normalizing FMD by the flow stimulus. Each treatment was done in the supine position for the entire FMD test and collection of blood flow velocities using the ultrasound.

Exercise

The exercise session lasted approximately 2 hours. Setting up ultrasound and measuring FMD took approximately 30 minutes before and 30 minutes after exercise training for a total of 1 hour of testing for FMD. Ten minutes of supine rest was done before FMD was measured. Exercise was done by performing plantar flexion for approximately 30 minutes. Progressively increasing intensity was done to determine and moderate intensity for the subject to perform for

20 minutes. Two minute intervals of 5psi increment increases were done until subjects and researcher agreed on a moderate workload for subject to perform for 20 minutes. Subject and researcher subjectively determined moderate intensity by choosing a load that would moderate for the entire 20 minutes. Exercise was done by performing plantar flexions every second for 5 seconds followed by 5 seconds of rest and was repeated at this pattern for a total of 20 minutes. Blood flow velocities was measured and captured during plantar flexions. After the 20-minute training, the subjects rested for 40 minutes until 1 hour has passed before measuring post-training FMD. Flow-mediated dilation was measured in the posterior tibial artery in the left leg prior and after treatment for all subjects. After the posterior artery was found initially, markers were place on the ankle to mark probe placement to minimize variability in artery measurement location and therefore minimizing artery diameter variability due to different locations. When measuring blood flow velocities, the ultrasound probe was places over the femoral artery of the left leg for all subjects. To maintain consistent measurements markers were placed on the left thigh to mark probe placement for each subject.

Compression Therapy

The compression therapy session lasted approximately 2 hours. Setting up ultrasound and measuring FMD lasted approximately 30 minutes before and 30 minutes after compression therapy for a total of 1 hour of testing for FMD. Ten minutes of supine rest was done before FMD was measured. The compression therapy device was turned on and ran for a minimum of 20 minutes prior to using the device on the subject to allow the device to warm up. Inflation leg garments were placed on both legs of the subjects while they were in the supine position on the lab table. Compression therapy consisted of 30 seconds of inflation with 5 seconds of deflation

of the leg garments for both legs reaching a maximum inflation pressure of ~60mmHg. Blood flow velocities were measured and captured during compression therapy treatment (during first 10 minutes, midpoint, and last 10 minutes of therapy). Total compression therapy time was 1 hour. After the one-hour compression therapy, the subjects were tested for post-training FMD.

Time Control

The time control session lasted approximately 2 hours. Setting up ultrasound and measuring FMD took approximately 30 minutes before and 30 minutes after one-hour time control for a total of 1 hour of testing for FMD. Subject was rested in the supine position for 10 minutes prior to FMD measurements were collected. Time control consisted of one-hour of sitting quietly in front of a lab computer which allowed for work or web surfing. Subjects were asked to sit as still as they could help. After the one-hour time control, the subjects were tested for post-treatment FMD.

OUTCOME MEASURES

The outcome variables that were measured were FMD and blood flow velocities in the left leg of all subjects. Both measurements were measured using a GE Logiq 400 ultrasound. Measurements were captured using Doppler and B-mode ultrasound. FMD was measured in the posterior tibial artery while blood flow velocities were measured in the femoral artery.

Flow-mediated Dilation

Flow-mediated dilation was measured using video capturing software in conjunction with ultrasound imaging. Measuring the diameter of the posterior tibial artery at rest and peak

diameter following peak reactive hyperemia following cuff occlusion was how FMD was assessed. The artery diameter was captured using B-mode ultrasound connected to a digital video capture device. Ten-second videos were recorded at rest in the posterior tibial artery. A vascular occlusion cuff was inflated proximal and across the knee joint from the site where the resting diameter was captured. The cuff was inflated to a pressure of 100mmHg over systolic blood pressure of the subject. The cuff stayed inflated for 5 minutes. Ten-second videos were captured at 4 and 4.5 minutes of cuff occlusion. Finally ten seconds before release of the cuff video were captured for a total of 2 minutes and 10 seconds of video to capture peak diameter after peak reactive hyperemia. The videos were decompiled into individual jpeg images using Blaze Media Pro software. From there, the images were analyzed to obtain mean diameter across a cardiac cycle using a MatLab Image Analyzer wall detection program. The Image Analyzer program output data was opened with Excel to get the needed data.

After baseline and peak diameter, which was taken after release of cuff and reactive hyperemic flow, was determined, FMD was calculated as follows:

(Peak diameter – Baseline diameter)/(Baseline diameter) *100 = FMD (%)

Blood Flow Velocities

Doppler OCR program was used to capture blood flow velocities during FMD measurements and during a period of time of exercise training/compression therapy treatment. Traditionally Pulsed Doppler ultrasound is recorded in the longitudinal view using an angle between 55 and 65 degrees, which is what was done in this study. Flow speed measurements that rely on Doppler shift measurements are only as accurate as the confidence of the Doppler angle of the measurement. Estimations are done by aligning the SV line on the anatomical display of

the ultrasound so that the line is parallel to the vessel walls. This subjective method has a higher chance of error at higher angles. Due to this reason, Doppler angle should not be greater than 60 degrees (35). Doppler Angle is also considered when analyzing diameters. Due to the diameter analyzing software, MatLab Image Analyzer, rotating the images (post decompiling) so that the walls are oriented to 90 degrees, there is less error when measuring diameters when Doppler angle is nearer to 90 degrees (28). With both of these error components being considered, it was decided to allow Doppler angles to exceed 60 degrees (recommendation for velocity measurements) in order to enhance diameter measurements. Careful monitoring of the exact angle was used to minimize error for the velocity measurements. The velocity gate was set to include the entire diameter of the vessel.

Statistical Analysis

Hypothesis testing was performed using a repeated measure ANOVA to measure a difference within and between treatments in the study.

Paired t-test was used to calculate the difference between pre and posttest FMD values. Statistical significance was set at $p \leq 0.05$. Values are expressed as mean ± SD unless stated otherwise. With the number of subjects in the study, 8, variability in measurement, 0.5, alpha = 0.05 and power = 0.8, an effect size of 0.76 could have been detected and a 38% change could have been detected.

Results

Subject Characteristics

Eleven subjects started and completed all 3 sessions of the study. We excluded 3 subjects from the data analysis because of bad data due to wall image quality and/or analysis failure. We used the remaining 8 subjects in our data analysis. Table 3.1 shows subject characteristics.

Blood Flow Velocities

Compared to baseline velocities, measured during supine rest, there was no significant increase in mean blood flow velocities over time observed during compression. During exercise, mean blood flow velocities over time was observed to be much greater than during baseline and compression therapy. However, when observing and measuring blood flow velocities, there were episodic increases in velocities when the compression therapy device was deflating (every 30 seconds). Exercise treatment also showed episodic increases in blood flow velocities in between reps of plantar flexion. When comparing episodic velocity peaks, compression therapy showed ~3 fold increase in velocity while exercise showed a ~9 fold increase. Figure 3.5 shows average peak velocities observed during specific treatments.

Flow-Mediated Dilation

Only data that fit criteria determined before start of study were used in data analysis and final results. These criteria were resting diameter within 5% difference of one another, peak diameter observed between the time of 30-100 seconds after release of cuff release, and diameter that decreased after peak diameter was observed were used in data analysis and results.

Flow-mediated dilation was not significantly different between or within treatment groups, $F = 0.128$, $p = 0.881$. Pre-post FMD values were 14.1±9.0% and 15.4±7.9% for exercise, 15.6±10.3% and 17.3±5.5% for compression, and 15.6±10.8% and 15.2±15.1% for control. Pre-post measurements of FMD were not significantly different in the control, compression, and exercise groups ($p=0.897$, 0.636, and 0.683 respectively). Figure 3.8 shows pre-post averages of all 8 subjects during control, compression, and exercise treatment groups. Based on the number of subjects, 8, variability in measurement, 0.5, alpha = 0.05 and power = 0.8, a 38% change could have been detected.

Discussion

This study was successful in measuring blood flow velocities in the femoral artery during baseline, compression therapy, and exercise in eight smokers. We found that total blood flow over time was not significantly increased with compression therapy compared to baseline. In fact, there could have been a slight decrease in total blood flow over time in compression therapy when compared to baseline. Total blood flow over time with exercise was much higher than observed during baseline. Gonzales et al. observed similar increases during fast and slow knee extensions (23). With compression therapy, there is not an increase in total blood flow over time, but it was observed there were episodic increases in peak blood flow velocities when the device was in the deflation stage (every 30 seconds). This episodic peak was ~3 folds higher than that observed during baseline. This was not as high as observed during exercise, which was ~9 fold higher than baseline, but it was significantly higher. This episodic increase in blood flow velocities, according to Green et al., (25-27) is important to vascular adaptations. Also it was observed that the magnitude of anterograde and retrograde was increased with compression

therapy. This could be important because of the debate on "pattern of flow" and how it influences vascular adaptations. Gonzales et al. makes the point that retrograde blood flow is a significant shear stimulus (23), while Green et al. stress the importance magnitude of anterograde and retrograde and "pattern of flow" on vascular adaptations (25, 26).

Total blood flow over time was not observed to be different between compression therapy and baseline could be due to the fact that the stimulus from the compression device decreased over time. When the device first arrived in the lab, it could reach a pressure of ~100 mmHg. By the time the subjects were participating in the study, it would only be able to reach pressures around ~65 mmHg. This inflation pressure could be too low to be a significant stimulus during treatment.

Few papers measure vascular reactivity in the lower limbs and even fewer are measured in the posterior tibial artery. Black et al. (2) looked at FMD in the posterior tibial artery in 6 subjects. The values for FMD they observed were 13.5±6.6% in the posterior tibial artery. When looking at all FMD values in our study we observe values of 15.5±9.7% across the sample population. Our values were close to those values seen in Black's paper. Another study reports FMD values much lower Gokce et al. (22) report values of ~10% with a standard deviation of approximately 2.1%.

The observed F value and t-test values showed that there was no significance between or within treatments during this study. An explanation for this is that the FMD measurements collected were simply too variable. FMD is usually done in the brachial artery and not in the lower limbs much less a small artery in the lower limb like the posterior tibial artery. Variability could be due to the variability in the technician, ultrasound imaging quality, data collection and analysis technique, and variability in the posterior tibial artery itself. Whatever the reason, the

FMD measurements were too variable to find small changes in FMD within or between treatments.

Our hypothesis was that compression therapy and/or exercise will improve FMD with a single bout of treatment, given that it is in an impaired population. The subjects in this study did not seem to have impaired FMD from the start. That could explain why there was no significance between pre and posttest for FMD with treatments. If there were no impairments in FMD, then it would be much more difficult to find improvements in FMD. Finally, while one bout of exercise and one bout of compression therapy did not change FMD, this does not exclude that more bouts could improve FMD.

Table 3.1 Subjects Characteristics

	Age	Height (cm)	Weight (kg)	BMI	Years Smoked
Mean	22.5	175.1	73.8	24.0	3.3
SD	2.5	6.5	12.9	3.8	1.9

n= 8; 7 males, 1 female, values are mean (SD).

FIGURE LEGENDS

Figure 3.1 Experimental flow chart. After consent procedures and orientation is done, one of the three treatments, randomly assigned, is performed after a 10 minute rest period and before another 10 minute rest period.

Figure 3.2 Compression therapy setup showing probe placement for velocity and FMD measurements

Figure 3.3 Representative exercise blood flow velocities. Average peak velocities are shown along with average resting velocities across the graph.

Figure 3.4 Representative compression therapy blood flow velocities. Values shown are averages of peak velocities during compression compared to resting velocities averaged.

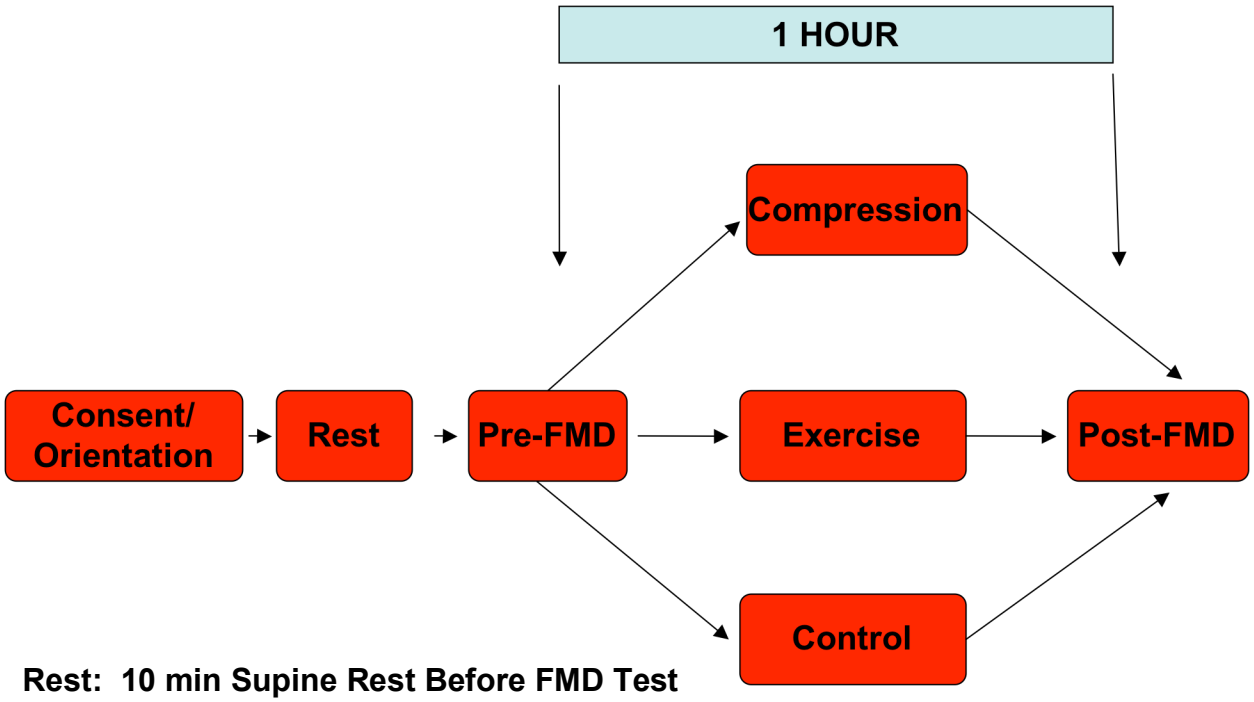
Figure 3.5 Average peak velocities with compression and exercise. Values shown are averages of peak velocities during compression and exercise treatment compared to resting velocities averaged. Error bars are SD.

Figure 3.6 Representative resting diameters. Graph shows resting diameter of resting subject prior to inflation of cuff for FMD measurement with another graph showing portion of rest with smaller scale to show cardiac cycle in the arterial walls.

Figure 3.7 Change in arterial diameter after cuff release. Graph shows arterial diameter after release of cuff during FMD measurements. First 10 seconds of graph is missing because that is the last 10 seconds of inflated cuff prior to release.

Figure 3.8 Pre-Post Flow-mediated dilation. Graph shows average FMD before and after treatment of exercise, control, and compression of 8 subjects. Error bars are in SD.

Figure 3.1 Experimental Flow Chart



Rest: 10 min Supine Rest Before FMD Test

Compression: 60 min

Exercise: Progressive Plantar Flexion, Followed by 20 min at Steady Intensity

Figure 3.2 Compression Therapy Setup

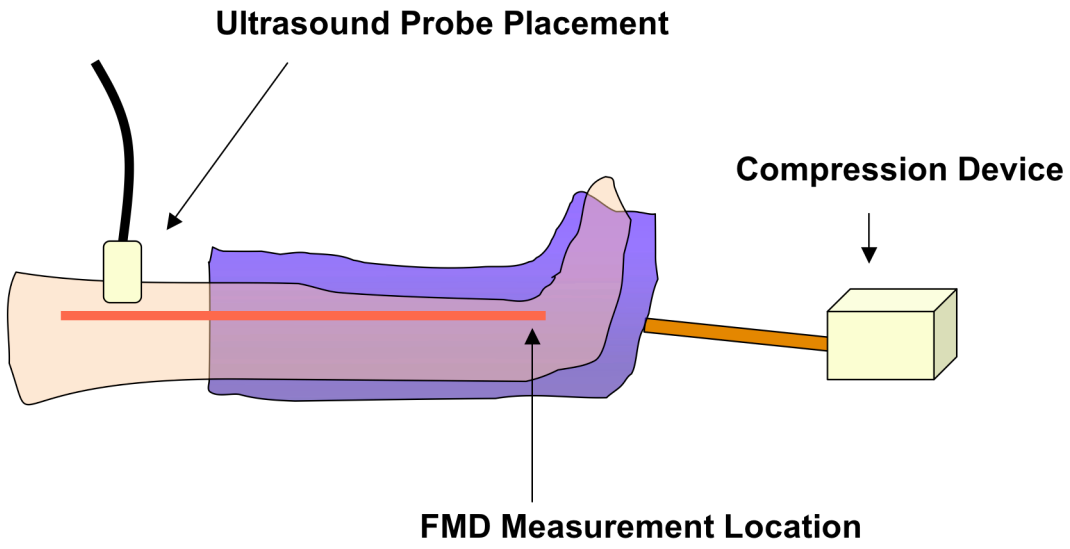


Figure 3.3 Representative Exercise Blood Flow Velocities

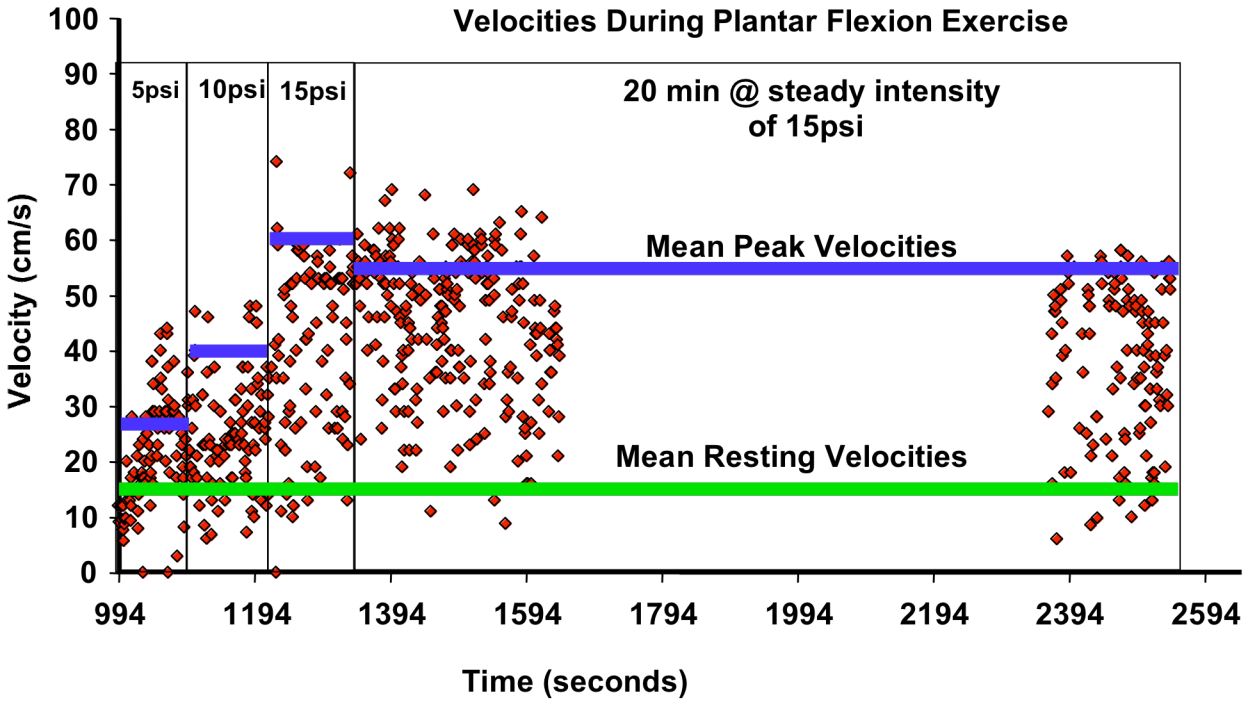


Figure 3.4 Representative Compression Therapy Blood Flow Velocities

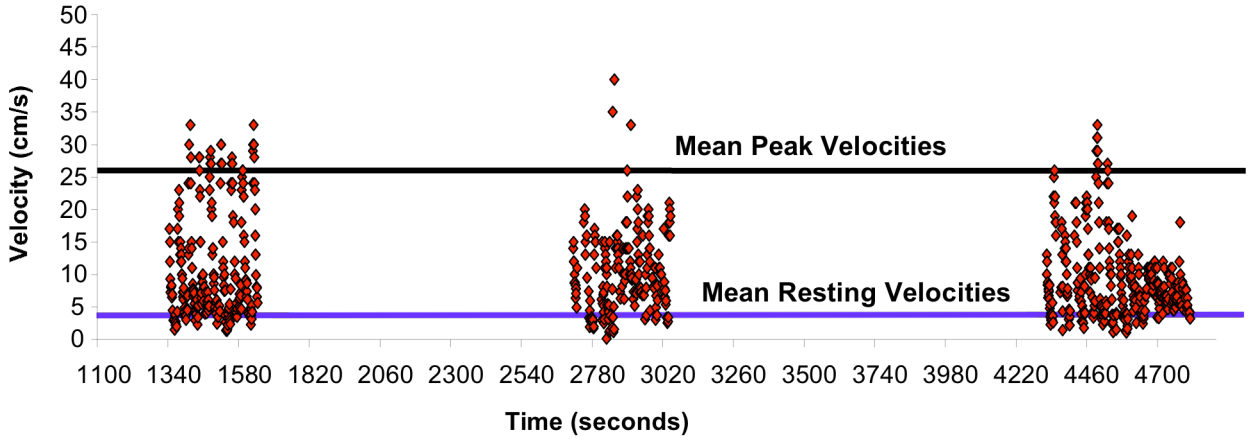


Figure 3.5 Average Peak Velocities with Compression and Exercise

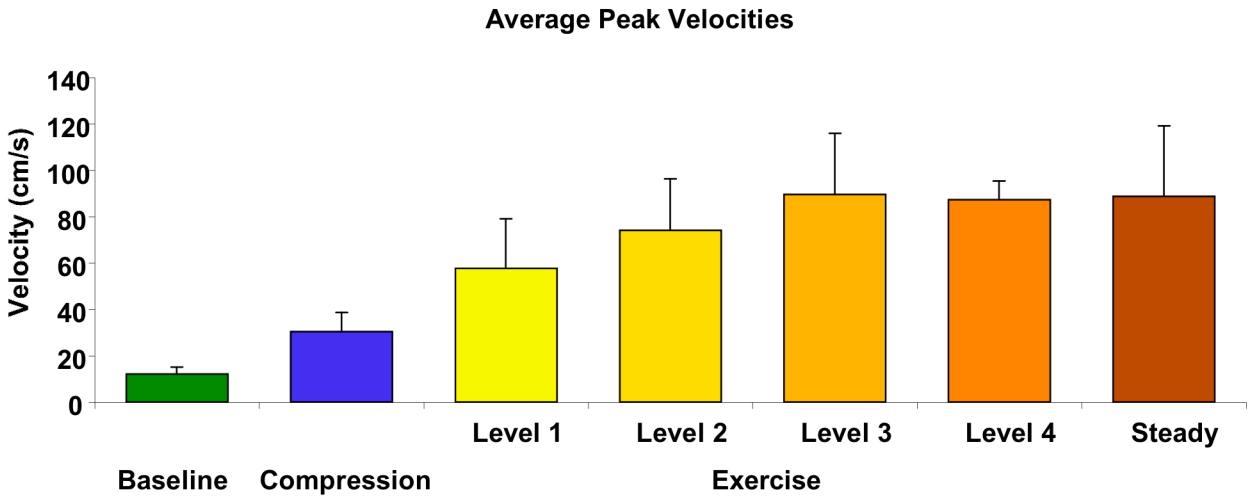


Figure 3.6 Representative Resting Diameter

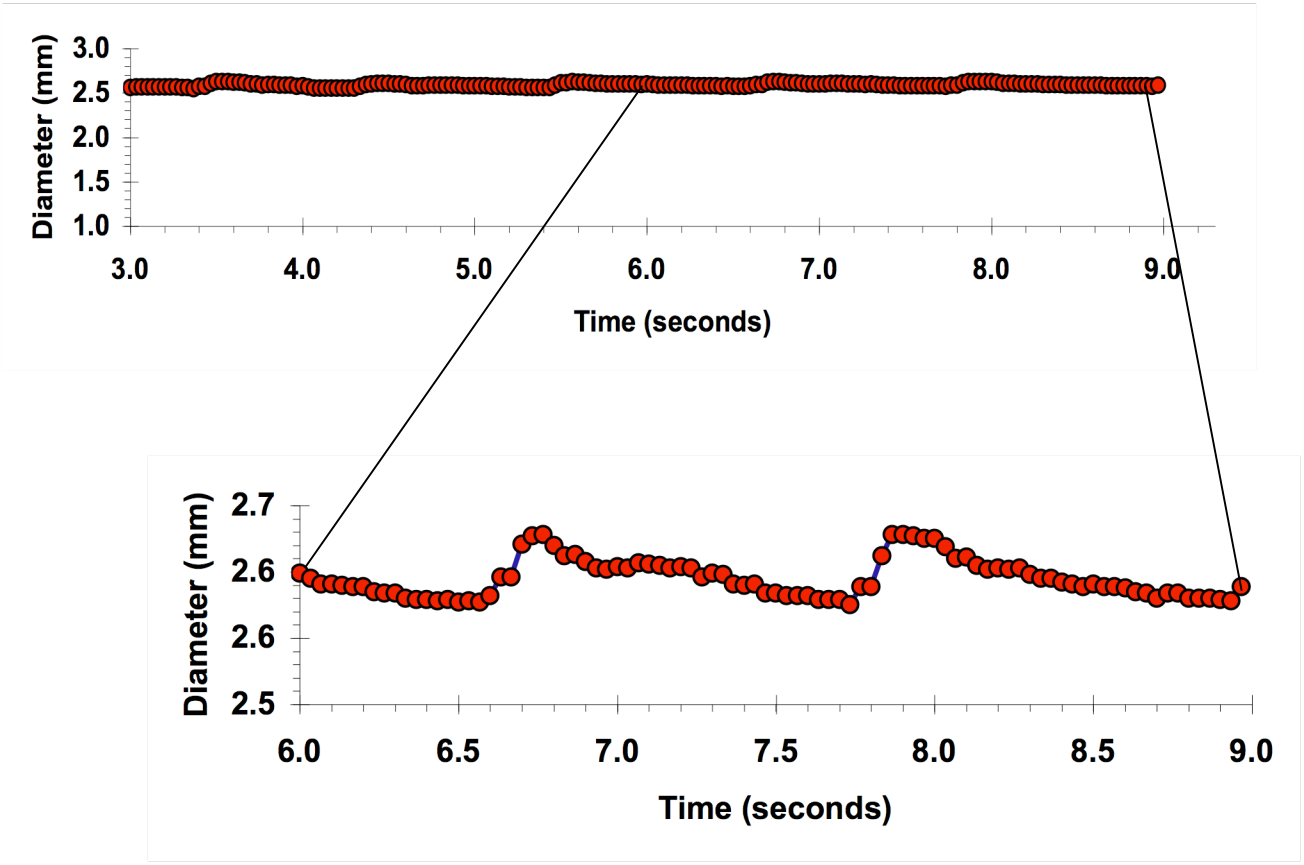


Figure 3.7 Change in arterial diameter after cuff release

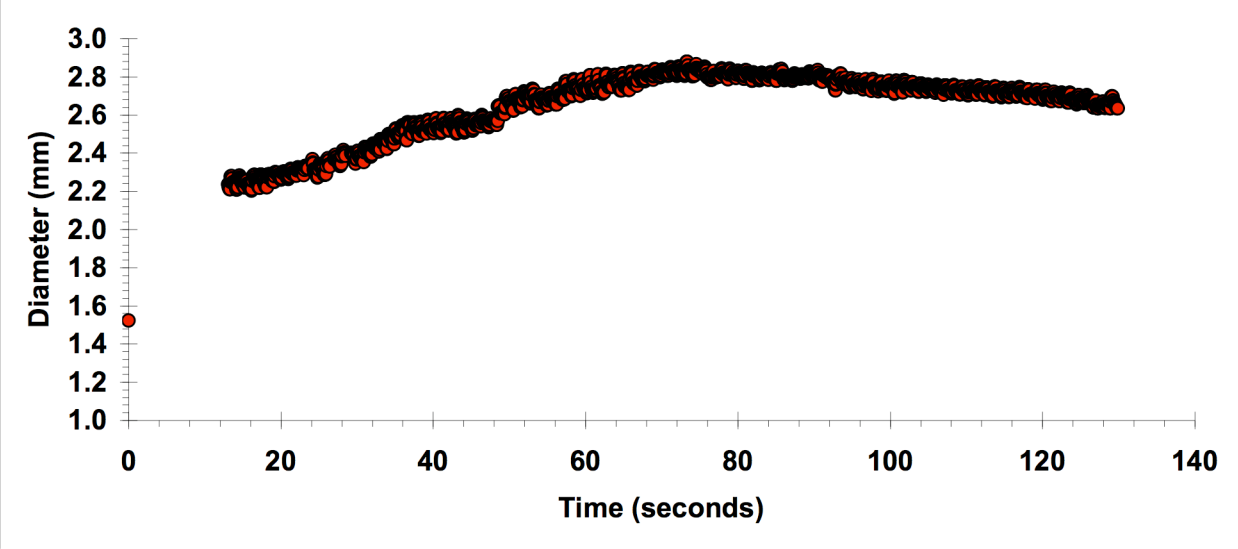
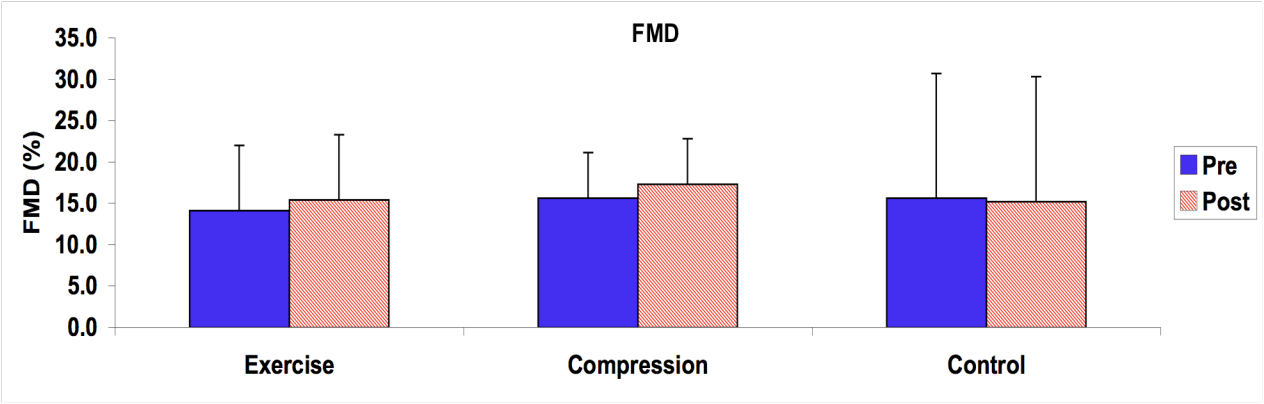


Figure 3.8 Pre-Post FMD



CHAPTER 4

Discussion, Summary, and Conclusions

While we were unable to find a difference between pre and posttest FMD with the treatments and between each treatment, we were able to measure and observe significant differences in blood flow between control, compression therapy and exercise. This study also found valuable information that will benefit future studies in this field, like differences in episodic blood flow spikes in velocity and considerations in pressures of compression therapy and its possible importance to stimulate blood flow increases.

This study took a looked further at the importance of pattern of flow and how this could affect vascular adaptation and endothelial health. It has been understood that when discussing FMD, with an increase in shear stimulus there is a nitric oxide-dependent vasodilatation of the smooth muscles of the arteries(18, 30, 37, 41). We were looking more closely into that "increase in shear stimulus". It has been stated by Green et al. (27) that pattern of flow should be important when considering the shear stimulus for FMD. Blood flow has a pulsatile nature of flow and not a steady flow pattern. This can be observed in the cardiac cycle where there are two distinctly different shear stimuli present. During systole, the blood flow a higher acceleratory shear stimuli while during diastole, there is a steadier shear stimuli. These two stimuli, observed in vitro, have been shown to regulate acutely and in a long-term manner endothelial function by different mechanical pathways (10, 16, 36). This rationale is important to consider when observing the blood flow response with compression therapy. Since the mean blood flow over time does not seem to increase, it could be simply argued that compression

therapy has no shear stimulus increase above that of rest. When considering pattern of flow and the acceleratory differences seen episodically with compression therapy, one could argue there is sufficient shear stimulus with treatments that change pattern of flow like compression therapy. In this particular study, the difference in shear stimulus was possibly too low to find a significant increase in FMD. Finally, negative flow seen during inflation stages of compression therapy increased. FMD not improving could have been affected by this increase in negative flow increase seen with compression therapy. This could help understand why flow-mediated dilation was not improved with compression therapy.

Compression therapy should not be abandoned because of the low increase in shear stimulus that was observed in this study. Compression therapy is still a safer and easier option than such methods as electrical stimulation, FES cycling, and assisted treadmill walking for certain clinical population, like SCI patients. To look more closely at the possibility of compression therapy increasing blood flow and FMD, the issue of acquiring a higher stimulus should be addressed. The low stimulus observed from the compression device has led to development of another compression device with a higher pressure and faster inflation/deflation times. This could lead to a stronger stimulus and therefore allow the researcher to test the hypothesis that compression therapy will increase blood flow and improve FMD in an impaired population. Valuable data was collected and observed for blood flow response due to compression therapy. With increased stimulus, there could be a greater chance that this alternative to exercise could have vascular adaptations that corresponds with the change in blood flow. The idea that compression therapy could be used as an alternative to exercise is very important to clinical populations that are unable to exercise, like spinal cord injury patients. This could have a great impact on their health and well-being.

Compression therapy is just one possibility that could increase the shear stimulus, which according to Green et al. (27) is the response observed during exercise that is beneficial to endothelial health and vascular adaptation. Compression therapy and external counter-pulsation therapy are just two of the possible many therapies that could increase shear stimulus to enable an improvement in FMD.

In conclusion, the hypotheses of compression therapy having similar blood flow velocity increases similar to that of exercise was not accepted and a single bout of exercise/compression therapy would improve FMD was not accepted.

CHAPTER 5

References

1. **Allen JD, Geaghan JP, Greenway F, and Welsch MA.** Time course of improved flow-mediated dilation after short-term exercise training. *Med Sci Sports Exerc* 35: 847-853, 2003.

2. **Black CD, Vickerson B, and McCully KK.** Noninvasive assessment of vascular function in the posterior tibial artery of healthy humans. *Dyn Med* 2: 1, 2003.

3. **Brevetti G, Silvestro A, Schiano V, and Chiariello M.** Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* 108: 2093-2098, 2003.

4. **Chan SY, Mancini GB, Burns S, Johnson FF, Brozic AP, Kingsbury K, Barr S, Kuramoto L, Schulzer M, Frohlich J, and Ignaszewski A.** Dietary measures and exercise training contribute to improvement of endothelial function and atherosclerosis even in patients given intensive pharmacologic therapy. *J Cardiopulm Rehabil* 26: 288-293, 2006.

5. **Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Crexler H, Gerhard-Herman M, Derrington D, Vallance P, Vita J, and Vogel R.** Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *Journal of the American College of Cardiology* 39: 257-265, 2002.

6. **Corretti MC, Plotnick GD, and Vogel RA.** Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. *Am J Physiol* 268: H1397-1404, 1995.

7. **Delis KT, Labropoulos N, Nicolaides AN, Glenville B, and Stansby G.** Effect of intermittent pneumatic foot compression on popliteal artery haemodynamics. *Eur J Vasc Endovasc Surg* 19: 270-277, 2000.

8. **Demopoulos L, Bijou R, Fergus I, Jones M, Strom J, and LeJemtel TH.** Exercise training in patients with severe congestive heart failure: enhancing peak aerobic capacity while minimizing the increase in ventricular wall stress. *J Am Coll Cardiol* 29: 597-603, 1997.

9. **Djaberi R, Beishuizen ED, Pereira AM, Rabelink TJ, Smit JW, Tamsma JT, Huisman MV, and Jukema JW.** Non-invasive cardiac imaging techniques and vascular

tools for the assessment of cardiovascular disease in type 2 diabetes mellitus. *Diabetologia* 51: 1581-1593, 2008.

10. **Duffy SJ, Castle SF, Harper RW, and Meredith IT.** Contribution of vasodilator prostanoids and nitric oxide to resting flow, metabolic vasodilation, and flow-mediated dilation in human coronary circulation. *Circulation* 100: 1951-1957, 1999.

11. **Esen AM, Barutcu I, Acar M, Degirmenci B, Kaya D, Turkmen M, Melek M, Onrat E, Esen OB, and Kirma C.** Effect of smoking on endothelial function and wall thickness of brachial artery. *Circ J* 68: 1123-1126, 2004.

12. **Eze AR, Comerota AJ, Cisek PL, Holland BS, Kerr RP, Veeramasuneni R, and Comerota AJ, Jr.** Intermittent calf and foot compression increases lower extremity blood flow. *Am J Surg* 172: 130-134; discussion 135, 1996.

13. **Fahs PS and Kalman M.** Matters of the heart: cardiovascular disease and rural nursing. *Annu Rev Nurs Res* 26: 41-84, 2008.

14. **Fathi R and Marwick TH.** Noninvasive tests of vascular function and structure: why and how to perform them. *Am Heart J* 141: 694-703, 2001.

15. **Flynn KE, Pina IL, Whellan DJ, Lin L, Blumenthal JA, Ellis SJ, Fine LJ, Howlett JG, Keteyian SJ, Kitzman DW, Kraus WE, Miller NH, Schulman KA, Spertus JA, O'Connor CM, and Weinfurt KP.** Effects of exercise training on health status in patients with chronic heart failure: HF-ACTION randomized controlled trial. *JAMA* 301: 1451-1459, 2009.

16. **Frangos JA, Huang TY, and Clark CB.** Steady shear and step changes in shear stimulate endothelium via independent mechanisms--superposition of transient and sustained nitric oxide production. *Biochem Biophys Res Commun* 224: 660-665, 1996.

17. **Fuchsjager-Mayrl G, Pleiner J, Wiesinger GF, Sieder AE, Quittan M, Nuhr MJ, Francesconi C, Seit HP, Francesconi M, Schmetterer L, and Wolzt M.** Exercise training improves vascular endothelial function in patients with type 1 diabetes. *Diabetes Care* 25: 1795-1801, 2002.

18. **Furchgott RF and Zawadzki JV.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376, 1980.

19. **Gaenzer H, Neumayr G, Marschang P, Sturm W, Kirchmair R, and Patsch JR.** Flow-mediated vasodilation of the femoral and brachial artery induced by exercise in healthy nonsmoking and smoking men. *J Am Coll Cardiol* 38: 1313-1319, 2001.

20. **Gerrits HL, de Haan A, Sargeant AJ, van Langen H, and Hopman MT.** Peripheral vascular changes after electrically stimulated cycle training in people with spinal cord injury. *Arch Phys Med Rehabil* 82: 832-839, 2001.

21. **Giannini D, Leone A, Di Bisceglie D, Nuti M, Strata G, Buttitta F, Masserini L, and Balbarini A.** The effects of acute passive smoke exposure on endothelium-dependent brachial artery dilation in healthy individuals. *Angiology* 58: 211-217, 2007.

22. **Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keaney JF, Jr., and Balady GJ.** Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol* 90: 124-127, 2002.

23. **Gonzales JU, Thompson BC, Thistlethwaite JR, and Scheuermann BW.** Role of retrograde flow in the shear stimulus associated with exercise blood flow. *Clin Physiol Funct Imaging* 28: 318-325, 2008.

24. **Granberry MC, Smith ES, 3rd, Troillett RD, and Eidt JF.** Forearm endothelial response in smokeless tobacco users compared with cigarette smokers and nonusers of tobacco. *Pharmacotherapy* 23: 974-978, 2003.

25. **Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G, and Walsh JH.** Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *J Physiol* 562: 617-628, 2005.

26. **Green DJ, Maiorana AJ, and Cable NT.** Point: exercise training does induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* 105: 1002-1004; discussion 1007, 2008.

27. **Green DJ, O'Driscoll G, Joyner MJ, and Cable NT.** Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol* 105: 766-768, 2008.

28. **Hoeks AP and Reneman RS.** Biophysical principles of vascular diagnosis. *J Clin Ultrasound* 23: 71-79, 1995.

29. **Hundley WG, Bayram E, Hamilton CA, Hamilton EA, Morgan TM, Darty SN, Stewart KP, Link KM, Herrington DM, and Kitzman DW.** Leg flow-mediated arterial dilation in elderly patients with heart failure and normal left ventricular ejection fraction. *Am J Physiol Heart Circ Physiol* 292: H1427-1434, 2007.

30. **Ignarro LJ.** Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ Res* 65: 1-21, 1989.

31. **Jodoin I, Bussieres LM, Tardif JC, and Juneau M.** Effect of a short-term primary prevention program on endothelium-dependent vasodilation in adults at risk for atherosclerosis. *Can J Cardiol* 15: 83-88, 1999.

32. **Karatzi K, Papamichael C, Karatzis E, Papaioannou TG, Stamatelopoulos K, Zakopoulos NA, Zampelas A, and Lekakis J.** Acute smoke-induced endothelial

dysfunction is more prolonged in smokers than in non-smokers. *Int J Cardiol* 120: 404-406, 2007.

33. **Kecelj Leskovec N, Pavlovic MD, and Lunder T.** A short review of diagnosis and compression therapy of chronic venous insufficiency. *Acta Dermatovenerol Alp Panonica Adriat* 17: 17-21, 2008.

34. **King N, Hopkins M, Caudwell P, Stubbs J, and Blundell J.** Beneficial effects of exercise: shifting the focus from body weight to other markers of health. *Br J Sports Med*, 2009.

35. **Kremkau F.** *Diagnostic Untrasound: Principles and Practice*. Philadelphia: W.B. Saunders Co., 1998.

36. **Kuchan MJ, Jo H, and Frangos JA.** Role of G proteins in shear stress-mediated nitric oxide production by endothelial cells. *Am J Physiol* 267: C753-758, 1994.

37. **Luscher TF and Barton M.** Biology of the endothelium. *Clin Cardiol* 20: II-3-10, 1997.

38. **Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, Taylor R, and Green D.** The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* 38: 860-866, 2001.

39. **McDermott MM, Ades P, Guralnik JM, Dyer A, Ferrucci L, Liu K, Nelson M, Lloyd-Jones D, Van Horn L, Garside D, Kibbe M, Domanchuk K, Stein JH, Liao Y, Tao H, Green D, Pearce WH, Schneider JR, McPherson D, Laing ST, McCarthy WJ, Shroff A, and Criqui MH.** Treadmill exercise and resistance training in patients with peripheral arterial disease with and without intermittent claudication: a randomized controlled trial. *JAMA* 301: 165-174, 2009.

40. **Michielli DW.** Invited commentary on viewpoint by Green et al.: Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol* 105: 769, 2008.

41. **Moncada S.** The L-arginine: nitric oxide pathway, cellular transduction and immunological roles. *Adv Second Messenger Phosphoprotein Res* 28: 97-99, 1993.

42. **Neunteufl T, Heher S, Kostner K, Mitulovic G, Lehr S, Khoschsorur G, Schmid RW, Maurer G, and Stefenelli T.** Contribution of nicotine to acute endothelial dysfunction in long-term smokers. *J Am Coll Cardiol* 39: 251-256, 2002.

43. **Olson TP, Dengel DR, Leon AS, and Schmitz KH.** Moderate resistance training and vascular health in overweight women. *Med Sci Sports Exerc* 38: 1558-1564, 2006.

44. **Partsch H, Flour M, and Smith PC.** Indications for compression therapy in venous and lymphatic disease consensus based on experimental data and scientific evidence. Under the auspices of the IUP. *Int Angiol* 27: 193-219, 2008.

45. **Quinton AE, Cook CM, and Peek MJ.** The relationship between cigarette smoking, endothelial function and intrauterine growth restriction in human pregnancy. *BJOG* 115: 780-784, 2008.

46. **Sandri M and Gielen S.** Comments on point: counterpoint: exercise training does/does not induce vascular adaptations beyond the active muscle beds. *J Appl Physiol* 105: 1008, 2008.

47. **Shechter M, Issachar A, Marai I, Koren-Morag N, Freinark D, Shahar Y, Shechter A, and Feinberg MS.** Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease. *Int J Cardiol*, 2008.

48. **Shechter M, Matetzky S, Feinberg MS, Chouraqui P, Rotstein Z, and Hod H.** External counterpulsation therapy improves endothelial function in patients with refractory angina pectoris. *J Am Coll Cardiol* 42: 2090-2095, 2003.

49. **Stoner L, Sabatier MJ, Black CD, and McCully KK.** Occasional cigarette smoking chronically affects arterial function. *Ultrasound Med Biol* 34: 1885-1892, 2008.

50. **Stoner L, Sabatier MJ, Mahoney ET, Dudley GA, and McCully KK.** Electrical stimulation-evoked resistance exercise therapy improves arterial health after chronic spinal cord injury. *Spinal Cord* 45: 49-56, 2007.

51. **Sung K.** The effects of 16-week group exercise program on physical function and mental health of elderly Korean women in long-term assisted living facility. *J Cardiovasc Nurs* 24: 344-351, 2009.

52. **Tabata I.** [Exercise, physical activities and public health (13). Amount of physical activities, exercise volume and physical strength that are necessary to prevent life-style-related diseases]. *Nippon Koshu Eisei Zasshi* 56: 184-187, 2009.

53. **Thijssen DH, Ellenkamp R, Smits P, and Hopman MT.** Rapid vascular adaptations to training and detraining in persons with spinal cord injury. *Arch Phys Med Rehabil* 87: 474-481, 2006.

54. **Thijssen DH, Heesterbeek P, van Kuppevelt DJ, Duysens J, and Hopman MT.** Local vascular adaptations after hybrid training in spinal cord-injured subjects. *Med Sci Sports Exerc* 37: 1112-1118, 2005.

55. **Thomas GN, Chook P, Yip TW, Kwong SK, Chan TY, Qiao M, Huang XS, Guo DS, Feng JZ, Chan SW, Leong HC, Celermajer DS, and Woo KS.** Smoking without

exception adversely affects vascular structure and function in apparently healthy Chinese: implications in global atherosclerosis prevention. *Int J Cardiol* 128: 172-177, 2008.

56. **Turner AP, Kivlahan DR, and Haselkorn JK.** Exercise and quality of life among people with multiple sclerosis: looking beyond physical functioning to mental health and participation in life. *Arch Phys Med Rehabil* 90: 420-428, 2009.

57. **Warden SJ and Fuchs RK.** Exercise and bone health: optimizing structure during growth is key, but all is not in vain during aging. *Br J Sports Med*, 2009.

58. **Woo KS, Chook P, Leong HC, Huang XS, and Celermajer DS.** The impact of heavy passive smoking on arterial endothelial function in modernized Chinese. *J Am Coll Cardiol* 36: 1228-1232, 2000.

59. **Yoshida T, Kawano H, Miyamoto S, Motoyama T, Fukushima H, Hirai N, and Ogawa H.** Prognostic value of flow-mediated dilation of the brachial artery in patients with cardiovascular disease. *Intern Med* 45: 575-579, 2006.