Abstract:

TRAINING AND ENDOTHELIAL FUNCTION IN UPPER AND LOWER LIMBS

Anna Kate Bires

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Director: Robert Hickner, PhD

Department of Kinesiology

Cardiovascular disease (CVD) begins with damage to the endothelium, the inner lining of the blood vessels. Endothelial damage occurs as early as childhood and is associated with risk factors such as obesity, hypertension, diabetes, smoking, and dyslipidemia. Previous research has documented that exercise enhances endothelial function in individuals of all ages, but there is no consensus in the literature as to whether this improvement is seen systemically or is localized to the exercised limb. It is well known that endothelial function is not homogenous throughout the body, due to differences in limb blood pressure when standing, which can be as high as 65 mmHg. The purpose of this study was to evaluate endothelial function of the brachial and popliteal arteries of trained and sedentary men via flow-mediated dilation (FMD). FMD is assessed by measuring changes in arterial diameter in the reperfusion period following a 5-minute period of ischemia. We hypothesized that 1) trained men would have greater endothelial function in both the brachial and popliteal arteries in comparison to sedentary counterparts 2) the brachial artery would have better endothelial function than the popliteal artery only in sedentary men. Brachial and popliteal endothelial function will be similar in trained men due to an exercise training improvement in endothelial function of the leg in trained individuals.

Baseline diameter (cm) and blood flow (ml/min) were measured in the brachial and popliteal arteries of 7 lower-body aerobically trained (T) and 7 sedentary (S) young, healthy

men. A blood pressure cuff was inflated to occlude the artery for 5 minutes. Upon releasing the cuff, diameter and blood flow were measured intermittently for 5 minutes via Doppler Ultrasound to evaluate the vessel's reactive hyperemic response. FMD was calculated relatively, as a percent change from pre-occlusion, and absolutely, as a cm change from pre-occlusion. The data were analyzed utilizing a 2X2 ANOVA and linear regression.

Trained men exhibited enhanced endothelial function in comparison to sedentary counterparts when FMD was expressed as a percent change (T brachial=15.12 ± 8.44%; S brachial =8.71 ± 2.57%; T popliteal= 8.35 ± 5.03%; S popliteal 5.24 ± 2.57%; p= 0.029) and as an absolute change (T brachial= 0.06 ± 0.03cm; S brachial 0.04 ± 0.01cm; T popliteal=0.05 ± 0.29cm; S popliteal 0.03 ± 0.02cm; p=0.013). In both groups, the brachial artery had better endothelial function than the popliteal artery (p=0.019).

In accordance with the hypothesis, endothelial function was enhanced in trained subjects compared to sedentary controls. However, contrary to our prediction, greater endothelial function was expressed in the brachial than popliteal arteries of both groups. It was concluded that aerobically trained subjects have an improved endothelial function as compared to sedentary subjects in the lower and upper body.

# TRAINING AND ENDOTHELIAL FUNCTION IN UPPER AND LOWER LIMBS

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By Anna Kate Bires

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TRAINING AND ENDOTHELIAL FUNCTION IN UPPER AND LOWER LIMBS

by

Anna Kate Bires

APPROVED BY:

DIRECTOR OF THESIS:_____
(Robert C. Hickner, Ph.D.)

COMMITTEE MEMBER:_____
(Timothy P. Gavin Ph.D.)

COMMITTEE MEMBER:_____
(Mike McCammon M.A.)

COMMITTEE MEMBER:_____
(Carol A. Witczak, Ph.D.)

CHAIR OF THE DEPARTMENT OF KINESIOLOGY:_____
(Stacey R. Altman, J.D., M.Ed.)

DEAN OF THE GRADUATE SCHOOL:_____
(Paul J. Gemperline, Ph.D.)

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# CHAPTER I: INTRODUCTION

Cardiovascular disease (CVD) has been the number one cause of death in the United States for the past fifty years.[1] Currently, 12 million Americans have coronary heart disease and 4 million have had a stroke.[1] Furthermore, the prevalence of CVD will likely increase as the United States population lives longer with chronic disease.[2] In fact, direct medical costs due to CVD are projected to triple from 275.5 billion to 818.1 billion dollars between 2010 and 2030.[3] CVD begins with damage to the endothelium. This damage begins as early as childhood, and is correlated with cardiovascular risk factors.[4] It is well documented that exercise can improve endothelial function in individuals of all ages.[5,6,7]

A process termed flow-mediated dilation (FMD) has been validated as a measure of endothelial function in conduit arteries.[8] This process measures arterial diameter during reactive hyperemia, an increase in blood flow following a period of ischemia. Several studies have shown that endothelial function in the brachial artery is positively correlated with endothelial function in the coronary arteries.[9,10] Thus, FMD may be utilized to evaluate endothelial function and detect the early stages of atherosclerosis.

It is widely accepted that endothelial function is not homogeneous throughout the systemic arteries.[11,12,13] This may be attributed to the blood pressure difference between the arms and legs when standing, which can be as high as 65mmHg. This blood pressure difference negatively affects the endothelium of the lower extremities, which initiates the process of atherosclerosis. This may explain why peripheral artery disease (PAD) is much more common in lower rather than upper extremities.[14] Therefore, performing FMD in the leg in addition to the arm may provide a better assessment of endothelial function.

Exercise improves endothelial function, but it is unclear whether aerobic exercise training improves endothelial function in both upper and lower extremities. To our knowledge, no study has investigated both brachial and popliteal FMD in trained and sedentary young men. Doing so will allow us to compare endothelial function in the upper and lower extremities in both trained and sedentary individuals.

*Purpose:*

The purpose of this study is to assess differences in flow-mediated dilation in the brachial and popliteal arteries in sedentary and trained young, healthy men.

*Hypothesis:*

We hypothesized that 1) brachial artery endothelial function would be better than popliteal endothelial function in sedentary men due to blood pressure differences when standing, which negatively effects the endothelium of the leg 2) trained men would have better endothelial function in both brachial and popliteal arteries as compared to sedentary men 3) there would be no significant differences between brachial and popliteal endothelial function in trained subjects due to an exercise training improvement in endothelial function of the leg of trained individuals.

# CHAPTER II: LITERATURE REVIEW

*Blood flow and the role of endothelium:*

Blood flow is the volume of blood moving through a vessel, organ, or entire circulation. It is equal to cardiac output, which is approximately 5.0 to 5.5 L/min at rest.[15] Opposition to blood flow, peripheral resistance, is affected by blood viscosity, vessel length and vessel diameter. Peripheral resistance increases with increasing length of the vessels, and decreases with increased vessel diameter.[15] Blood flow is directly proportional to the difference in blood pressures between two points; as the difference in blood pressures increase, so does blood flow.[15] An inverse relationship exists between blood flow and peripheral resistance. Resistance is altered via vasoconstriction or vasodilatation.[15] Blood flow is autoregulated to maintain tissue's blood requirements at any instant through metabolic and myogenic mechanisms.[15]

Endothelium was once thought to simply be a lining of blood vessels, however, it is now understood that endothelial cells are responsible for maintaining homeostasis through physical, chemical, and humoral mechanisms.[16] Normal endothelium regulates vascular tone, prevents platelet adhesion and aggregation, has antithrombotic properties, and controls vascular growth.[17] Vascular tone is maintained by the release of biologically active substances from endothelial cells. Some of these substances include prostacyclin, endothelium-derived relaxing factor (EDRF), known as nitric oxide, and endothelial-derived hyperpolarizing factors. These chemicals induce vasodilation of the artery through relaxation of the smooth muscle cells.[16] Constricting agents include arachidonic acid metabolites and endothelin-1.[16] The endothelium has the ability to initiate both angiogenesis and the abnormal growth of smooth muscle cells in the presence of disease. Nitric oxide inhibits both of these processes.[17]

When endothelium is damaged, the cells do not function properly. Anticoagulant, antithrombotic, anti-inflammatory, and dilatory responses are compromised allowing for the buildup of plaque.[15] Endothelium damage is associated with the following: increasing age, male sex, dyslipidemia, obesity, stress, diabetes, sedentary lifestyle, cigarette smoking, hypertension, and a family history of atherosclerosis.[15] After endothelial cells are damaged, lipids accumulate on the arterial walls. This allows foam cell build-up and the formation of fatty streaks, which progress to atherosclerotic plaques.[15] It is widely accepted that the process of atherosclerosis begins early in life. A study conducted by the Pathobiologic Determinants of Atherosclerosis in Youth (PDAY) studied the arteries of 1,532 individuals aged 15 to 35 for fatty streaks and raised lesions.[4] The study concluded that early lesions were present in all individuals aged 15 to 19 and there was a strong correlation between cardiovascular risk factors and lesions.[4] Furthermore, several studies have shown that endothelial function is compromised in obese children compared to their lean counterparts.[6,18] This demonstrates that before atherosclerosis is detectable, vessels are diseased to a degree even in adolescents.[19] Therefore, it is feasible to prevent cardiovascular disease through modification of risk factors in children and adolescents. A noninvasive method of testing endothelial function has been in practice for approximately twenty years. As medicine shifts to a more preventative approach, it may be reasonable to incorporate this new method of testing to identify individuals with endothelial dysfunction.

*Flow-mediated dilation:*

Functional endothelial cells release nitric oxide (NO), a powerful vasodilator, in response to shear stress.[20] Shear stress is the tangential pull on the endothelial cells as blood flows through the artery; it is directly proportional to the viscosity of blood.[21] As the endothelial cells release NO, they send signals to the tunica media, the smooth muscle of the artery, to relax and promote

vasodilation. Vasodilators in the smooth muscle cause a series of reactions that decrease the calcium concentration and relax the artery's smooth muscle, resulting in vasodilation. This process is termed flow-mediated dilation (FMD). FMD is an indicator of vascular health as it reflects endothelium-dependent function.[12] A normal FMD response in the brachial artery is up to a 20 percent increase in diameter.[8]

FMD is affected by several factors including dietary or alcohol intake, recent aerobic or resistance exercise, supplement or medication use including oral contraceptives and hormone replacement therapies, time of day, and room temperature.[12] Thijssen et al. recommends that subjects abstain from these activities for at least six hours prior to being tested, and that there be a standardized time and room temperature for measurements to be taken to eliminate these extraneous variables.[12]

Celermajer et al. devised a method to test FMD by using high-resolution ultrasound to measure the diameters of the brachial and femoral arteries of individuals at rest. To evaluate endothelial dependent dilation, the conduit artery was occluded, and the diameter was measured after a period of ischemia. Celermajer et al. also tested endothelium independent dilation, by administering sublingual glyceryl trinitrate, a drug that releases NO. Upon the release of NO, the artery was occluded and arterial diameter was observed, allowing the function of the intima media (smooth muscle) to be evaluated.[8] Currently the use of high-resolution ultrasounds is a valid and reliable method of measuring endothelial function.[22,23] An alternative way to determine endothelial-dependent function is to infuse acetylcholine into the perspective artery and observe dilation. This drug stimulates the release of nitric oxide, thus it tests endothelial-dependent function. Conversely, sodium nitroprusside is injected to detect endothelial-independent

dilation. This drug releases nitric oxide to allow endothelial-independent function to be assessed.[10]

*Limb Differences and arterial size:*

It is well documented that endothelial function is not uniform throughout the body in healthy individuals.[5,11,12,13] Each artery supplies blood to a specific part of the body; thus arteries differ due to the demands of tissues they supply. The arteries in the legs are larger in diameter as compared to those in the arm because they supply larger muscle groups and achieve a four-to-eight fold increase in blood flow during exercise.[13] Blood flow of the upper extremities increases four fold with leg exercise. A study by Calbet et al. assessed blood flow in the arms and legs in response to cycle ergometry.[24] It was determined that leg vascular conductance was five-to-six times greater in comparison to arm vascular conductance. The blunted vascular conductance in the arm is a result of vasoconstrictor signals that oppose vasodilatory metabolies. The contracting muscles of the leg are less sensitive to this sympathetic response, which increases with exercise to maintain blood pressure.[24] Thus, there is greater dilation and blood flow in the legs as compared to the arms during upright cycle ergometry. Furthermore, the arms and legs have very different contributions during lower leg exercise. Calbet et al. determined that leg work during cycle ergometry was responsible for 84% of whole body $VO_2$ max, while the non-contracting arms contribute just 7-10% of whole body $VO_2$ max.[24] This may partially explain why upper body FMD is not predictive of lower body FMD.[12] There is a correlation between the dominant and non-dominant limbs of the same artery.[12] Therefore, we would expect no significant difference in FMD between the subject's dominant and non-dominant arm or leg.

Yet another reason for non-uniform endothelial function is the difference in blood pressure between the upper and lower limbs in an upright posture, which can be as high as

65mmHg.[14] This difference negatively affects the endothelial cells in the legs, allowing the lower extremities to be more susceptible to plaque build-up.[14] A study by Newcomer et al. demonstrated this by infusing acetylcholine and sodium nitroprusside to test endothelial dependent and independent responses respectively, into the brachial and femoral arteries of healthy, young men.[5] The study concluded that responses to the drugs were lower in the leg as compared to the forearm.[5] Thus, it appears evident that pressure differences negatively affect the endothelium of the lower body. Consequently, peripheral artery disease (PAD) is much more common in lower extremities than the upper extremities.[14]

Furthermore, FMD is inversely related to artery size. Smaller arteries experience greater shear stress than larger arteries during reactive hyperemia.[25] The femoral artery is on average twice as large as the popliteal artery, thus it is less reactive to NO in comparison to the popliteal.[26] Therefore, the current study will determine differences between brachial and popliteal FMD, as they are more similar in size and they are located similarly in the vascular system relative to their limbs.[26]

It is important to report FMD as an absolute change in diameter and as a relative change, or percent change.[22] Baseline diameter accounts for 15 percent of the variance in percent change, while it only accounts for 0.8 percent of the variance in absolute change in diameter.[25] Also, resting artery diameter is correlated with time to peak dilation.[22] The slightly smaller brachial artery will reach peak diameter in response to reactive hyperemia more quickly than the larger popliteal artery.

*FMD as an indicator of endothelial dysfunction in individuals with coronary artery disease:*

Several studies have found a positive relationship between endothelial function in the brachial and coronary arteries.[9,10] Studies by Anderson et al. and Takase et al. assessed

endothelial dependent and independent function of the brachial and coronary arteries.[9,10] Patients with normal endothelial function demonstrated dilation in their coronary arteries, while patients with endothelial dysfunction experienced vasoconstriction in response to administered acetylcholine.[10] Patients with endothelial dysfunction in the coronary arteries displayed a decreased FMD in the brachial artery.[10] There was a positive correlation ($r = 0.78$) between brachial artery FMD and coronary artery endothelial function.[9] Therefore, upper extremity endothelial function can be considered a "barometer" of cardiovascular health, and can be measured noninvasively via FMD.[27] While there is an established relationship between coronary and brachial endothelial function, there is no evidence that this relationship can be determined by measuring FMD in the lower limb.[12]

The presence of CVD, increased age, increased body mass index, and dysfunctional coronary artery endothelium are independent predictors of adverse cardiac events.[28,29,30,31] Cardiac events include death by cardiac causes, congestive heart failure, and coronary artery bypass graft.[29] Furthermore, the likelihood of future cardiac events can be determined by assessing endothelial function in patients with CVD.[27] Abnormal endothelial function is evident in the brachial arteries of individuals less than 40 years old with CVD.[30] As Americans are developing CVD at younger ages, FMD may be utilized as a non-invasive way to earlier detect disease in individuals at risk for cardiovascular disease. It may provide a way to save valuable health care dollars.

*FMD and exercise:*

Exercise increases blood flow, and consequently shear stress. Thus, exercisers experience chronic increases in nitric oxide, which has a positive effect on endothelial cells.[7,32] Endothelial function can be improved with exercise at all ages. Woo et al. demonstrated that

endothelial dysfunction was partially reversed with diet and exercise in obese children.[6] Similarly, Wray et al. determined that endothelial function improved in older men (72 $\pm$ 2 years) after participation in exercise.[33] These studies verify that endothelial function can be improved regardless of age.

It is well known that exercise training improves endothelial function of the active limbs.[32] A study by Franke et al. evaluated brachial artery endothelial function after four weeks of handgrip training. Participants engaged in handgrip training at seventy percent of maximum voluntary contraction.[34] Forearm vascular conductance, measured via strain-gauged plethysmography of the brachial artery, improved by thirty five percent.[34] Furthermore, a study by Dinenno et al. demonstrated that arterial remodeling is specific to the trained limb.[35] Participants engaged in three months of aerobic leg exercise training, mostly walking and jogging at 65 to 80 percent of their maximum heart rate.[35] Brachial and femoral diameter, intima media thickness, and tangential wall stress were measured via ultrasound. Femoral diameter was larger, had a decreased intima media thickness, and higher tangential wall stress in trained subjects compared to the sedentary subjects. There were no differences in the brachial artery between groups.[35] These studies demonstrate that limb specific exercises improve endothelial function of the trained limb.

Although exercise has been shown to improve endothelial function in the trained limb, there are conflicting data as to whether exercise training improves endothelial function uniformly throughout the body. A review by Maiorana et al. stated that improvements in endothelial function due to exercise can be reflected in untrained limbs due to the systemic response in blood flow when relatively large amounts of muscle mass are activated[32]however, several studies have challenged this hypothesis. Studies by Kingwell et al. and Desouza et al. demonstrated

endothelial improvement in the brachial artery due to aerobic exercise.[36,37] Participants in Kingwell's study engaged in four weeks of cycle training, which predominantly utilizes the legs. However, the non-trained vascular beds in the forearm showed improvement in endothelial function and increased forearm blood flow in response to lower leg exercise training.[36] This evidence suggests that elevated shear stress occurs in the brachial vascular bed during aerobic exercise, which may contribute to endothelial adaptations.[36]

Conversely, studies by Koller et al. and Jasperse et al. compared the arterial diameters and endothelial function of trained and untrained rats.[38,39] It was determined that exercised rats had greater arterial diameters and better endothelial function in the active vessels, but there was no significant difference in the passive vessels between trained and untrained rats.[38,40] Koller et al. determined that short-term daily exercise increases active vessel's sensitivity to shear stress. This increases nitric oxide production and vasodilatory response, which positively affects endothelial function of the active limb, but does not affect the inactive limb.[38] These results were seen in humans as well. A study including individuals with risk factors for atherosclerosis determined that brachial artery FMD was not significantly different before or after aerobic exercise training, which predominantly involved the legs.[40] Endothelial function of the lower extremities was not measured. This study demonstrates that aerobic training may have no effect on the endothelial function of inactive limbs.

The conflicting results of the aforementioned studies demonstrate that there is no consensus in the literature as to whether exercise training improves endothelial function in both upper and lower extremities, or if it is limb specific. Kingwell's study, which consisted of lower body exercise, demonstrated improvement in non-active muscular beds, while studies by Koller et al. and Jasperse et al. found no improvement in non-active muscle beds.[36,38,39]

Supporting Kingwell's results, a study by Clarkson et al. showed endothelial improvement in non-trained limbs. Subjects of this study were both aerobically and anaerobically trained. The training regimen consisted of three-mile runs in addition to upper and lower body resistance training.[7] Endothelial function of the brachial artery improved in trained subjects, but was unchanged in untrained subjects. However, like Kingwell's study, endothelial function of the leg was not measured. It is not clear as to whether the improvements in brachial endothelial function were solely attributed to upper body resistance training, or if leg aerobic training also played a role. Research including a comprehensive assessment of endothelial function is necessary to determine how exercise affects the upper and lower limbs of the body. Therefore, it would be advantageous to measure and compare endothelial function in both limbs of lower body aerobically trained and sedentary.

*Conclusion:*

Endothelial function can serve as a barometer for cardiovascular health. Therefore, FMD may provide a noninvasive method to assess individuals for the beginnings of CVD at young ages. It is understood that endothelial function is not uniform throughout the body. This may be attributed to blood pressure differences when standing, which negatively affects endothelial cells in the lower extremity. Thus it would be advantageous to measure both upper and lower limbs to better gauge one's endothelial function. While aerobic exercise has been shown to improve endothelial function, it is unclear if this improvement is seen in upper and lower extremities. By measuring the endothelial function of brachial and popliteal arteries in both trained and sedentary men, we can determine how exercise affects the endothelial function of both limbs.

# CHAPTER III: METHODS

Prior to testing, approval of methods was obtained by the University and Medical Center Institutional Review Board.

*Subjects*

Fourteen young men, aged 18 to 40 years, were recruited to participate in this study. Seven men were trained, defined as exercising for at least 30 minutes three or more days per week, and seven men were sedentary. Sedentary was defined as those who exercise no more than one day per week, and who have not participated in a structured exercise program in the past two months. Exclusion criteria included: obesity (BMI > 30); hypertension; a "Yes" response to any questions on the PAR-Q; smoker; and a history of cardiovascular disease. In order to quantify training status, trained participants were excluded if their $VO_2$ max was below the $75^{th}$ percentile for their age and sex. Sedentary participants were excluded if their $VO_2$ max was above the $50^{th}$ percentile for their age and sex. All subjects met this criterion, and no participants were excluded from the study based on their $VO_2$ max. Trained subjects were also excluded if they participated in upper body resistance training or sports that predominately utilizes the upper extremities.

*Instruments*

Body composition was assessed using dual energy X-ray absorptiometry (DEXA; GE Lunar Prodigy Advance, Madison, WI). A Hokanson Cuff Inflator and an ACUSON Sequoia 512 Doppler Ultrasound were utilized to assess FMD. Maximal oxygen consumption was measured via ParvoMedics TrueMax 2400 Metabolic Measurement cart during the treadmill test.

*Testing Protocol*

The testing of the subjects was covered in one visit. After the informed consent was reviewed and signed, height, weight, and body composition was measured. Body composition, and body mass, measured via DEXA scan, was used to calculate fat-free mass (FFM) and fat mass (FM). FFM and FM were also assessed specific to the subject's upper arm and calf.

Subjects were asked to fast for $\geq$ 6 hours prior to FMD testing. Subjects rested for 10 minutes prior to testing.[22] Baseline diameter and blood flow were measured intermittently for two minutes. The cuff was placed distal to the artery and inflated to 250 mmHg for 5 minutes.[22] Diameter and blood flow measurements were assessed four minutes into the occlusion to ensure the artery was occluded. After cuff deflation, vessel diameter and blood flow were measured immediately and intermittently, every minute, for five minutes thereafter.

One trained participant demonstrated constriction in response to reactive hyperemia. Thus, this subject's popliteal FMD was omitted from the analysis due to measurement error. FMD was presented as an absolute change (in cm) and as a relative change (in %).[22] Absolute FMD was calculated as: Peak diameter (cm) – Baseline diameter (cm), while relative FMD was calculated as: [Peak diameter (cm) – Baseline diameter (cm)] * 100%/Baseline diameter (cm). The day-to-day variability of FMD testing in this laboratory was 0.98 percent (relatively) and 0.01 cm (absolutely). Mean arterial pressure (MAP) was estimated and conductance (1/resistance) was calculated from MAP and artery blood flow.

Lastly, subjects performed a maximal exercise treadmill test to assess exercise capacity. The treadmill exercise test was designed to fatigue the subject within 8 to 12 minutes. The treadmill protocol for trained subjects began at 7.0 mph and progressed to 9.0 mph, with a two percent increase in grade every two minutes. The protocol for sedentary subjects began at 6.0

mph and progressed to 7.0 mph, with a two percent increase in grade every two minutes. Heart rate was monitored throughout the test. Subjects were asked to exercise until volitional fatigue. A treadmill test was considered maximal if the following criterion are met: 1) a heart rate in excess of 90% of age predicted max (220-age); 2) a respiratory exchange ratio (RER) greater than or equal to 1.10; and 3) identification of a plateau (<150ml increase) in $VO_2$ despite a further increase in workload.

*Statistical Analysis*

A student's T-test and two by two ANOVA were utilized to compare differences between groups. Linear regression was used to investigate associations between variables. Significance was established as $P \leq 0.05$, and data was reported as the Mean ± SD.

# CHAPTER IV: RESULTS

*Subjects*

Subject characteristics are outlined in *Table 1.* There were no significant differences in age, height, weight, BMI and MAP between trained and sedentary subjects, however a significant difference in body fat was detected.

*Flow Mediated Dilation (FMD)*

Trained subjects had significantly higher FMD, expressed as a percent change, than sedentary counterparts. In both groups, relative FMD was significantly higher in the brachial artery than the popliteal (*Figure 1).* However, there were no interactions. Similarly, trained subjects had significantly higher absolute FMD (cm change) than sedentary subjects. In contrast to relative FMD, there were no differences between sites. Again, there were no interactions (*Figure 2).* Both groups reached peak dilation in the brachial and popliteal artery one-minute post occlusion (*Figures 3 & 4).* There were no correlations between brachial and popliteal FMD in sedentary or trained men (*Figure 5).* There were no significant between group differences in baseline, resting diameter of the brachial or popliteal artery (*Table 1).* There were also no differences in baseline, resting diameter when adjusted to FFM of the upper arm or calf *(Table 1).* There were no correlations between resting blood flow and FMD, peak blood flow and FMD, brachial FMD and $VO_2$, popliteal FMD and $VO_2$, or body fat and FMD.

*Blood Flow and Conductance*

*Table 2* shows the averages in resting and peak blood flow and resting and peak conductance for trained and sedentary groups by site. There were no differences between groups or sites in resting or peak blood flow (*Figures 6 & 7).* Similarly, there were no differences between groups or sites in resting or peak conductance (*Figures 8 & 9).* The percent change in

conductance, from rest to maximum, revealed no significant between groups differences (*Table 2)*. In accordance with the absolute findings, there were no between groups differences in resting blood flow and conductance or peak blood flow and conductance when values were expressed relative to the limb's FFM (*Table 2).*

Trained subjects had significantly more variability in the relationship between resting blood flow and FMD in comparison to sedentary subjects (*Figure 10).* There were no significant interactions. Similarly, greater variation was seen in trained subjects compared to sedentary in the relationship between peak blood flow and FMD (*Figure 11).* Again, there were no significant interactions.

| | Trained (N = 7) | Sedentary (N = 7) | P Value |
|---|---|---|---|
| Age (yr) | 30.0 ± 5.9 | 23.3 ± 5.9 | 0.06 |
| Height (in) | 70.4 ± 2.01 | 72.1 ± 2.71 | 0.22 |
| Weight (lbs) | 160.8 ± 33.6 | 180.9 ± 29.8 | 0.26 |
| Mean Arterial Pressure (mmHg) | 86.5 ± 9.3 | 90.8 ± 8.9 | 0.39 |
| Body Composition | | | |
| BMI | 22.8 ± 4.4 | 24.5 ± 3.3 | 0.44 |
| Body Fat % | 14.0 ± 8.8 | 25.7 ± 9.4 | 0.03 |
| $VO_2$ max | | | |
| Absolute (L/min) | 3.90 ± 0.55 | 3.21 ± 0.72 | 0.03 |
| Relative (ml/min) | 54.1 ± 4.5 | 39.1 ± 1.1 | 0.003 |
| Resting Arterial Diameter | | | |
| Brachial (cm) | 0.42 ± 0.04 | 0.42 ± 0.03 | 0.20 |
| Relative to upper arm FFM (cm/kg) | 0.23 ± 0.05 | 0.20 ± 0.02 | 0.87 |
| Popliteal (cm) | 0.63 ± 0.09 | 0.61 ± 0.05 | 0.54 |
| Relative to calf FFM (cm/kg) | 0.31 ± 0.06 | 0.30 ± 0.05 | 0.75 |

*Table 1:* Subject Characteristics. BMI - Body Mass Index, $VO_2$max – maximal oxygen consumption. Values are expressed as Means ± SD.

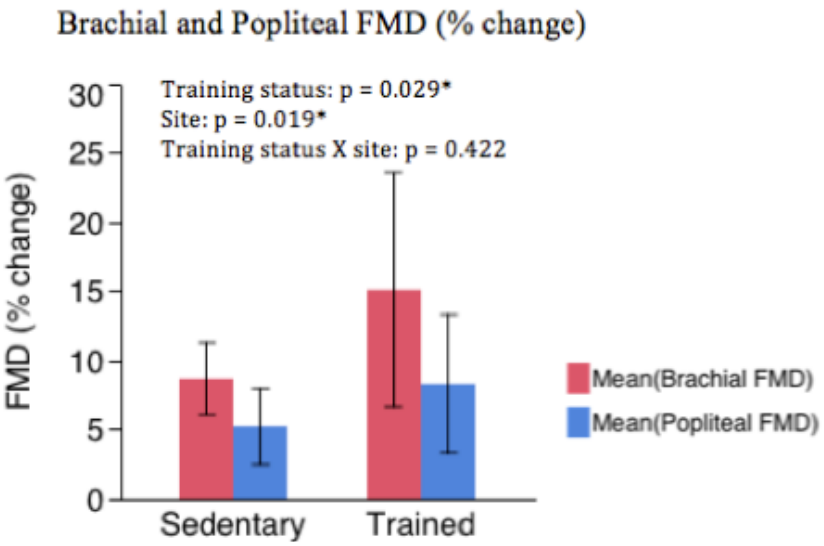



*Figure 1:* Brachial and popliteal flow mediated dilation (FMD) as a percent change from baseline in sedentary (n=7) and trained (n=6) groups. * indicates a significant difference between groups. Values are expressed as the mean ± SD.

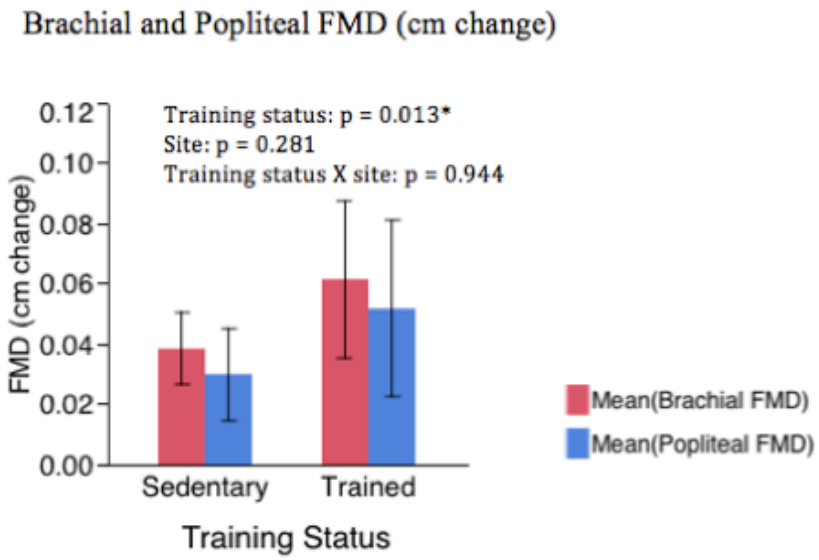



*Figure 2:* Brachial and popliteal flow mediated dilation (FMD) as a cm change from baseline in sedentary (n=7) and trained (n=6) groups. * indicates a significant difference between groups. Values are expressed as the mean ± SD.

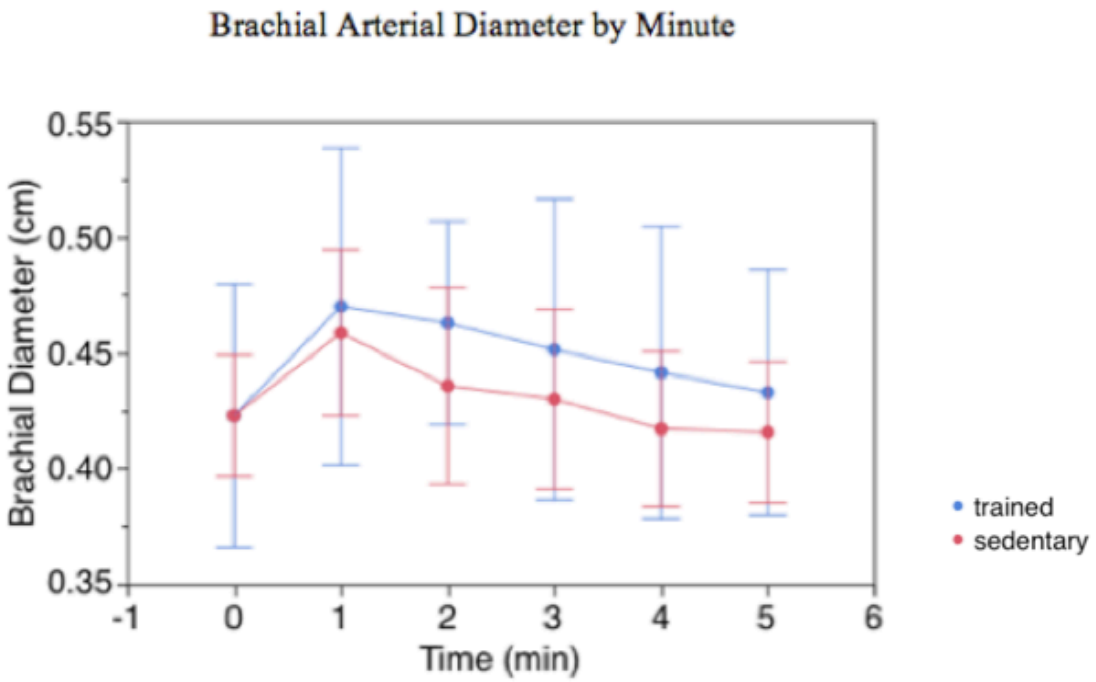



*Figure 3:* Brachial diameter (cm) at rest (0 min) and post-occlusion (1-5 min) in trained (n=7) and sedentary (n=7) subjects. Values are expressed as mean ± SD.

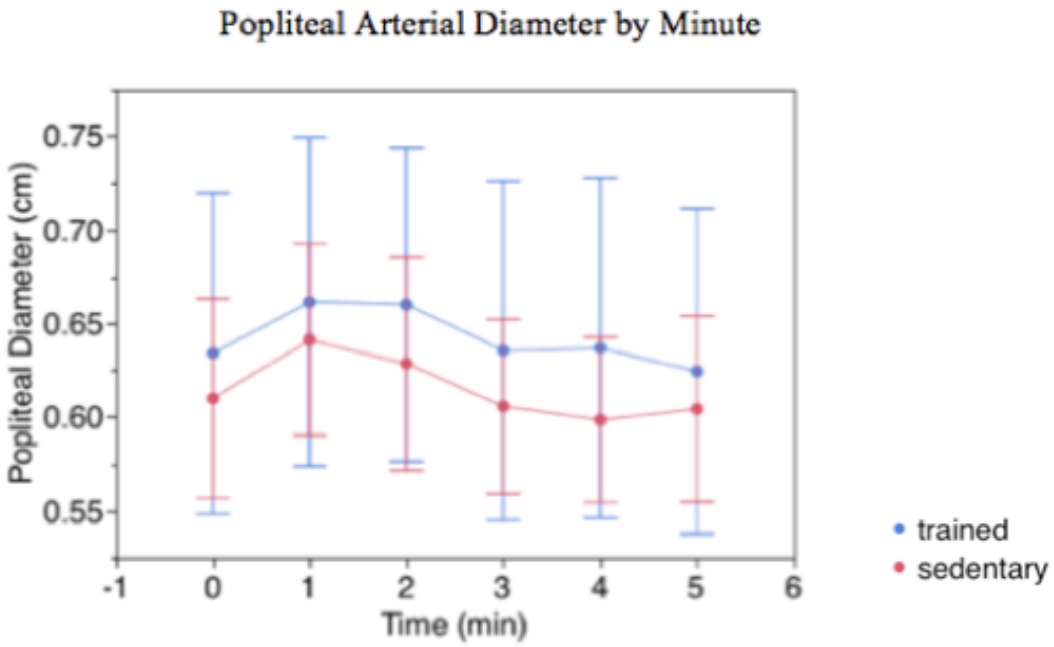
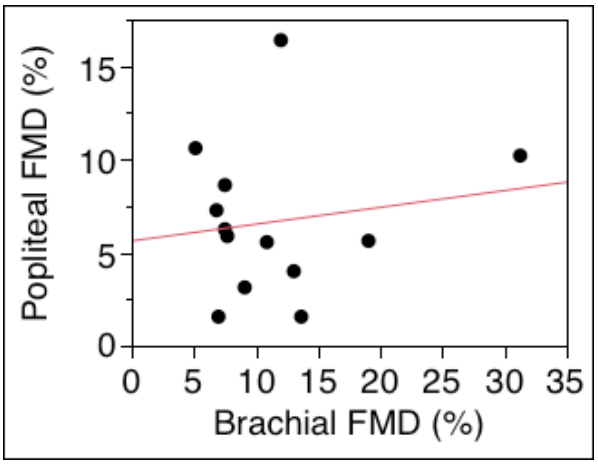
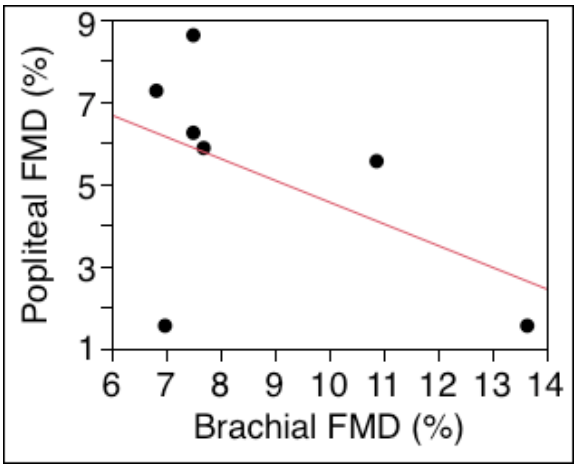



*Figure 4:* Popliteal diameter (cm) at rest (0 min) and post-occlusion (1-5 min) in trained (n=6) and sedentary (n=7) subjects. Values are expressed as mean ± SD.

5A.




5B.

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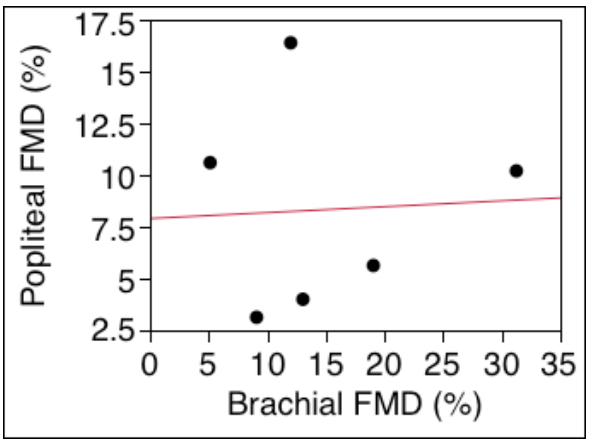



*Figure 5:* 5A. Brachial vs. popliteal FMD in trained (n=6) and sedentary (n=7) men (r=0.02). 5B. Brachial vs. popliteal FMD in sedentary men (n=7) (r=0.25). 5C. Brachial vs. popliteal FMD in trained men (n=6) (r=0.003).

| | Trained (N = 7) | Sedentary (N = 7) | P Value |
|---|---|---|---|
| Resting Blood Flow | | | |
| Brachial (ml/min) | 41.0± 32.2 | 68.3± 35.2 | 0.16 |
| Relative to upper arm FFM ($ml*100g^{-1}*min^{-1}$) | 22.3± 19.7 | 31.6± 15.4 | 0.35 |
| Popliteal (ml/min) | 70.8± 40.4 | 67.5± 42.9 | 0.88 |
| Relative to calf FFM ($ml*100g^{-1}*min^{-1}$) | 34.2± 18.2 | 32.2± 18.6 | 0.84 |
| Peak Blood Flow | | | |
| Brachial (ml/min) | 278.3± 121.6 | 308.7± 149.1 | 0.68 |
| Relative to upper arm FFM ($ml*100g^{-1}*min^{-1}$) | 153.4± 78.7 | 146.9± 69.7 | 0.87 |
| Popliteal (ml/min) | 349.5± 153.9 | 326.7± 167.3 | 0.80 |
| Relative to calf FFM ($ml*100g^{-1}*min^{-1}$) | 179.2± 101.5 | 163.4± 75.9 | 0.75 |
| Conductance | | | |
| Resting Brachial (U) | 0.46± 0.31 | 0.68± 0.34 | 0.20 |
| Peak Brachial (U) | 3.20± 1.39 | 3.37± 1.54 | 0.84 |
| % Change | 791.3± 564.0 | 558.3± 491.8 | 0.43 |
| Relative to upper arm FFM (U/kg) | 0.25± 0.19 | 0.32± 0.16 | 0.45 |
| Resting Popliteal (U) | 0.81± 0.43 | 0.74± 0.45 | 0.77 |
| Peak Popliteal (U) | 4.07± 1.74 | 3.52± 1.54 | 0.54 |
| % Change | 675.5± 833.3 | 519.6± 408.6 | 0.67 |
| Relative to calf FFM (U/kg) | 0.39± 0.20 | 0.35± 0.19 | 0.72 |

*Table 2:* Blood flow characteristics for brachial and popliteal arteries. Values are expressed as Means ± SE.

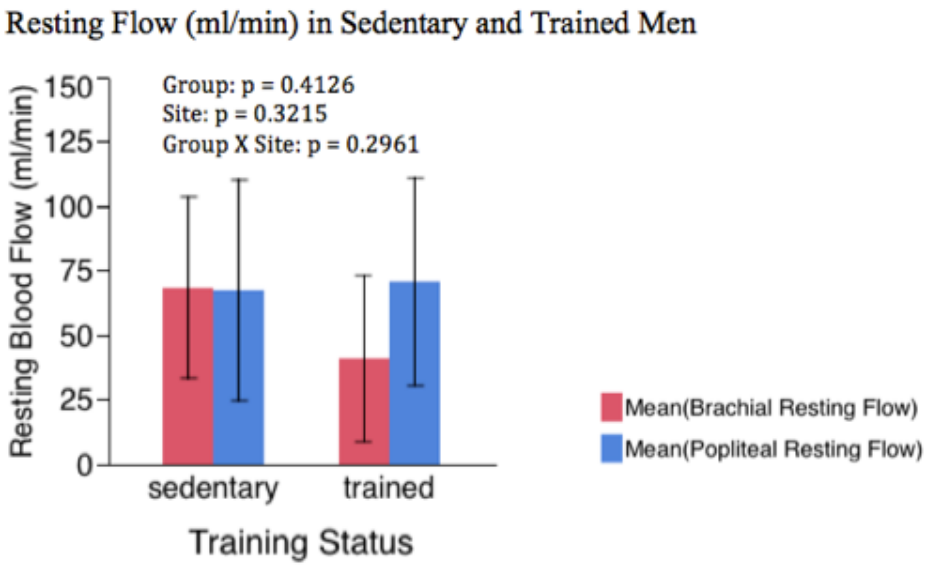



*Figure 6:* Resting brachial and popliteal blood flow in trained (n=7) and sedentary (n=7) men. * indicates significant differences. Values are expressed as mean ± SD.

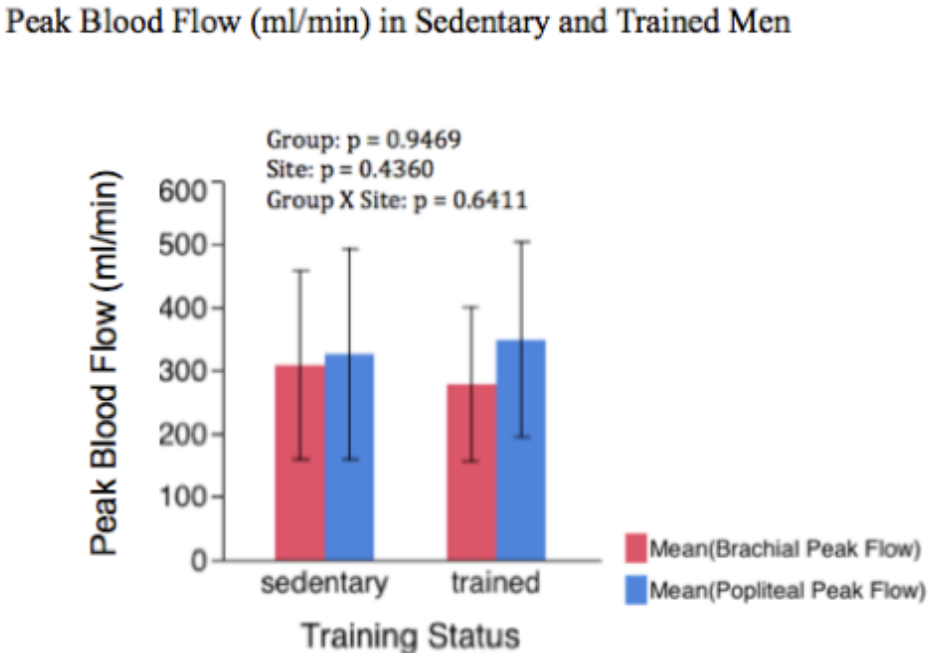



*Figure 7:* Peak brachial and popliteal blood flow in trained (n=7) and sedentary (n=7) men. * indicates significant differences. Values are expressed as mean ± SD.

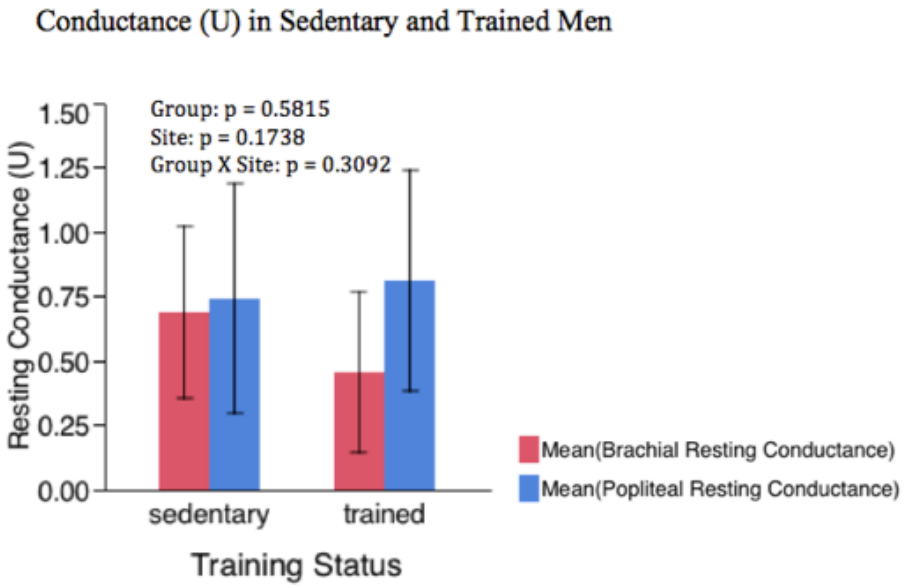



*Figure 8:* Resting brachial and popliteal conductance in trained (n=7) and sedentary (n=7) men. * indicates significant differences. Values are expressed as mean ± SD.

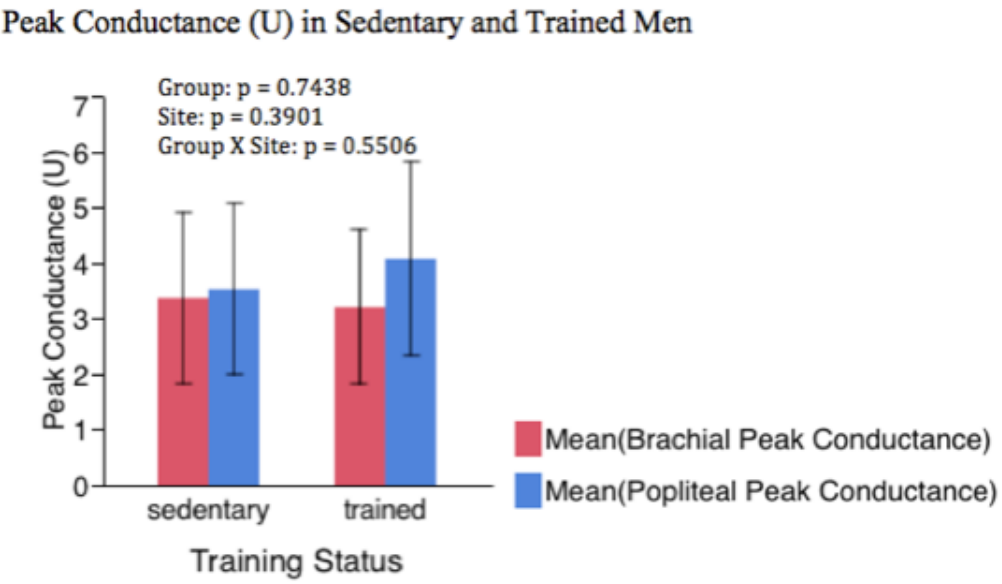
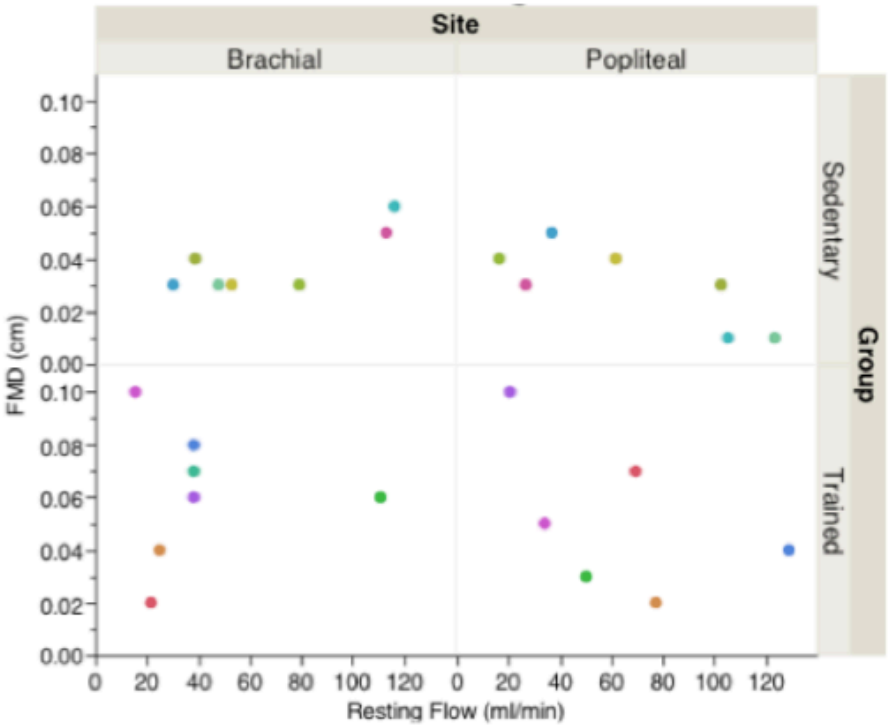
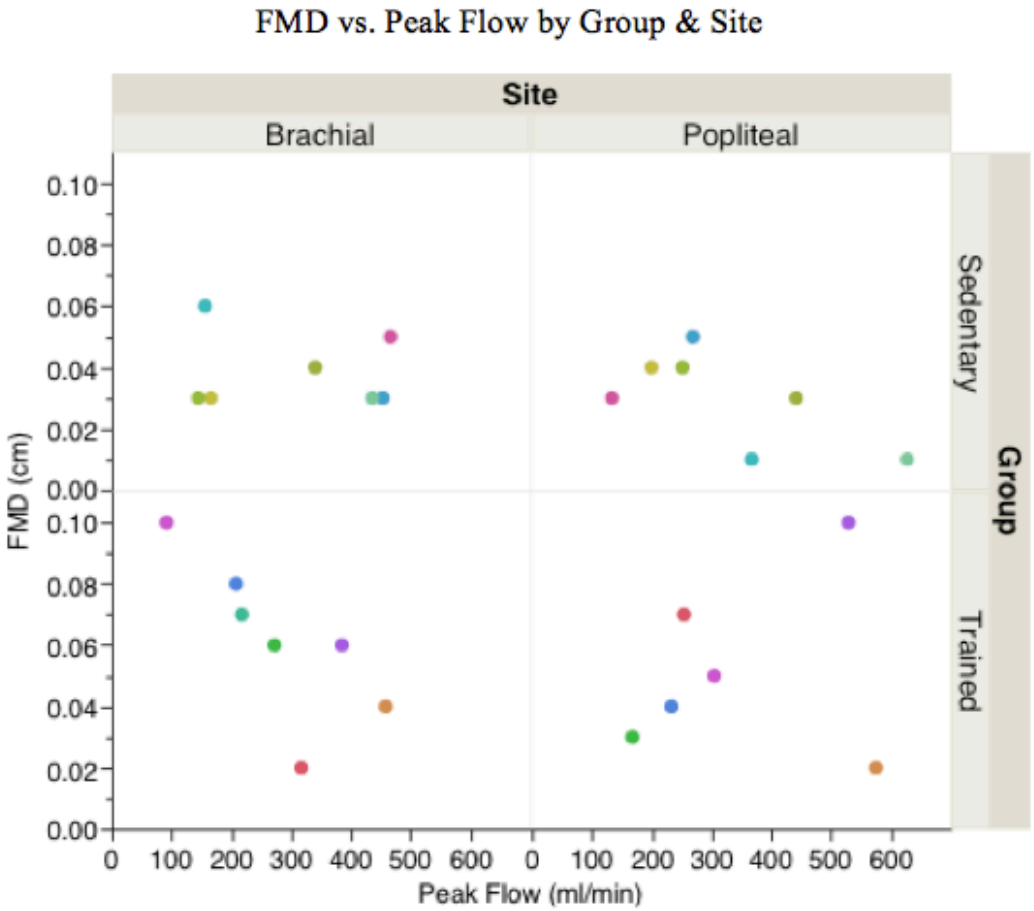



*Figure 9:* Peak brachial and popliteal conductance in trained (n=7) and sedentary (n=7) men. * indicates significant differences. Values are expressed as mean ± SD.




| Source of Variation | P Value |
|---|---|
| Resting Flow | 0.41 |
| Group | 0.01* |
| Site | 0.39 |
| Group *Site | 0.79 |
| Group * Resting Flow | 0.44 |
| Site * Resting Flow | 0.05 |

*Figure 10:* FMD (cm change) and resting blood flow (ml/min) in the brachial and popliteal arteries of trained (n=7) and sedentary (n=7) men. * indicates a statistical significance. Data for each subject is shown by multi-colored dots.




| Source of Variation | P Value |
|---|---|
| Peak Flow | 0.17 |
| Group | 0.02* |
| Site | 0.40 |
| Group *Site | 0.93 |
| Group * Peak Flow | 0.90 |
| Site * Peak Flow | 0.37 |

*Figure 11:* FMD (cm change) and peak blood flow (ml/min) in the brachial and popliteal arteries of trained (n=7) and sedentary (n=7) men. * indicates a statistical significance. Data for each subject is shown by multi-colored dots.

# CHAPTER V: DISCUSSION

In accordance with our hypothesis, this study demonstrated that trained men had better endothelial function than their sedentary counterparts in both brachial and popliteal arteries. Relative FMD, expressed as a percent change, showed significant differences between brachial and popliteal arteries in both groups. However, absolute FMD expressed as a change in arterial diameter (cm), was not significantly different between brachial and popliteal arteries in either group. Thus, our hypothesis that only sedentary men would have better endothelial function in the brachial artery than the popliteal artery was rejected. Both trained and sedentary groups were similar in age, height, weight, MAP, and BMI, although they differed in body fat percentage.

*Flow Mediated Dilation:*

This study found that trained subjects have better endothelial function, demonstrated by FMD, than their sedentary counterparts. In addition, relative FMD revealed better endothelial function in the brachial artery than the popliteal in both groups. These results demonstrate that 1) trained individuals exhibit enhanced endothelial function in comparison to sedentary counterparts 2) aerobic exercise training improves endothelial function systemically, demonstrated by greater endothelial function in both the brachial and popliteal arteries in trained subjects as compared to sedentary subjects.

It is well documented that aerobically trained individuals exhibit greater endothelial function than sedentary controls.[6,32–34] The novel finding in the current study is that aerobically trained men exhibit better endothelial function than sedentary men in the popliteal artery. Previous studies compared endothelial function of the brachial and femoral arteries, which are much different in size. To our knowledge, this study was the first to evaluate endothelial function of the popliteal artery in young, healthy trained and sedentary men. This allows for better

comparison of systemic vascular function as the brachial and popliteal are more similar in diameter and location in the vascular beds.[26]

There is no consensus in the literature, however, as to whether enhanced function is seen systemically or is limited to the exercised limb. The results of this study are in accordance with studies by Kingwell et al., Clarkson et al., and Desouza et al. Kingwell et al. reported increased endothelial function of the brachial artery in young men after four weeks of cycle ergometry training.[36] However, in contrast to this study, lower body endothelial function was not assessed. Clarkson et al. also demonstrated increased endothelial function of the brachial artery after ten weeks of an aerobic and anaerobic training program.[7] Participants in this study participated in upper body lifting in addition to a running regimen. Thus, it was unclear whether increases in brachial endothelial function were a result of upper body lifting or aerobic training. The current study attempted to eliminate this extraneous variable by excluding individuals that participate in upper body weights. Therefore, any improvements in brachial endothelial function are attributed to aerobic training. Trained participants in Desouza et al.'s study also showed increased brachial endothelial function. Participants were runners, and it was not stated that participants refrained from utilizing upper body weights.[37]

Conversely, Jasperse et al. and Jodoin et al. do not support the hypothesis that aerobic training lends to systemic improvements in endothelial function. Jasperse et al. report that the effects of exercise training on endothelial function are specific to the exercised limb in rat models.[39] In accordance, Jodoin et al. found no improvement in brachial endothelial function in humans in response to lower body aerobic exercise training.[40] The current study challenges these results, and supports the hypothesis that aerobic training improves endothelial function in untrained limbs. This is believed to result from increased systemic blood flow and large

increases in muscle mass recruitment with exercise.[32] The non-contracting arms have a four-fold increase in blood flow in response to cycle ergometry,[24] this increase in blood flow causes an increase in shear stress and NO bioactivity, and consequently dilation. Therefore, leg aerobic training may have a positive impact on the untrained upper limbs.

Contrary to our prediction, both groups experienced greater endothelial function in the brachial than the popliteal artery. Rather, an exercise training effect was seen in both limbs. Trained men showed greater leg and arm endothelial function in comparison to sedentary controls. Thus, it cannot be concluded that aerobic training improves the negative effects of blood pressure when standing. Studies by Malhotra et al. report that the lower limbs experience higher blood pressures than the arm. The pressure difference is 65mmHg higher on average. These blood pressure differences negatively affect the endothelium in the lower extremities and predispose individuals to peripheral arterial disease. While aerobic training improves lower body endothelial function, this study showed that it is significantly blunted in comparison to upper body endothelial function regardless of training status. In both trained and sedentary groups, brachial FMD was greater than popliteal FMD. Thus, the hypothesis that brachial FMD would be greater in comparison to popliteal FMD in only sedentary men was not supported. Rather, brachial FMD was superior to popliteal FMD in both groups.

In accordance to findings by Thijssen et al.,[12] the current study found no correlation between brachial and popliteal FMD in sedentary men. The current study determined that there is no correlation in endothelial function in upper and lower limbs in men who participate in aerobic training.

*Arterial Diameter:*

The literature suggests that aerobic athletes have larger diameters and thinner intima thickness due to arterial remodeling.[41] In contrast, this study found no significant differences in either brachial or arterial diameter between trained and sedentary males. This held true when arterial diameter was evaluated relative to the limb's FFM. Arterial remodeling is thought to occur in trained limbs due to repetitive periods of increased shear stress.[41] A study by Rowley et al. demonstrated that elite canoe paddlers and wheel chair athletes had larger brachial arterial diameters in comparison to controls while runners and cyclists had larger superficial femoral arteries compared to controls.[42] While Rowley et al.'s study suggested arterial remodeling in the exercised limb of elite athletes, the results of this study did not concur. We found no between groups differences in arterial size between the brachial or popliteal artery. The literature provides evidence that decreased arterial wall thickness is found in the peripheral blood vessels of aerobic athletes, in both exercised and non-exercised limbs.[43] Increased arterial wall thickness, like endothelial dysfunction, is a precursor to atherosclerosis.[44] Furthermore, it is understood that dysfunctioning endothelium initiates this abnormal smooth muscle growth in blood vessels.[17] Intima thickness in peripheral arteries can be reduced with aerobic exercise, but coronary arteries are less affected.[44] Thus, it may be feasible that aerobic exercise prevents atherosclerosis systemically by decreasing intima thickness of peripheral arteries in addition to improving endothelial function. However, unlike endothelial function,[10] a decreased intima thickness in the periphery is not an indicator of intima thickness in the coronary arteries.

*Blood flow and conductance characteristics:*

There were no between groups differences in resting blood flow or conductance in either the brachial or popliteal arteries. Also, there were no differences when resting blood flow and

conductance were expressed relative to limb FFM in trained and sedentary men. These results are consistent with a study by Snell et al., which evaluated resting blood flow and conductance of the brachial artery in aerobically trained and sedentary men.[45] In accordance with this study, Snell at al. found no between group differences in resting blood flow or resting conductance. However, in contrast, trained subjects in Snell et al.'s study exhibited significantly higher peak blood flow and conductance in comparison to sedentary counterparts.[45] Similarly, a study by Sinoway et al. demonstrated an increase in peak blood flow of exercised limbs of tennis players. Peak blood flow in the dominant arm was 42 percent higher than the nondominant arm.[46] This occurrence was not a result of aerobic fitness, as there was no difference in maximal oxygen consumption between groups. The results of this study challenge the results of Snell et al. and Sinoway et al.'s study. This study did not detect any significant between groups differences in peak blood flow or conductance in either the brachial or popliteal arteries. This held true when peak blood flow and conductance were expressed relative to limb FFM.

In the current study, trained subjects had greater variability in comparison to sedentary subjects in the relationship between resting blood flow and FMD. Trained individuals had greater variation in this relationship; a high resting flow was not equated with a high FMD and vice versa. Whereas sedentary individuals tended to have less variation, lower flow was associated with lower FMD and vice versa. This same relationship was also demonstrated in the variability between peak blood flow and FMD. It may seem intuitive that a higher peak blood flow would create higher shear stress, greater release of NO, and greater dilation. However, this was not the case in the current study, as some individuals had low peak blood flow and high FMD, while others had very high peak blood flow and low FMD. Peak blood flow was not correlated with greater endothelial function.

*Future Studies:*

Many unknowns remain regarding exercise training and endothelial function. This study evaluated multiple participants that were highly trained runners, running between 30 to 70 miles per week, with $VO_2$ maxes above the $75^{th}$ percentile for their age and sex. Future studies should include standardized training programs where participants meet the minimum guidelines for exercise according to the American College of Sports Medicine. If differences in endothelial function can be detected between moderately trained and sedentary groups, it will provide yet another reason why moderate exercise is an integral component in preventing the beginnings of atherosclerosis and peripheral artery disease.

*Conclusions:*

The novel findings of this study were that 1) aerobically trained males have better endothelial function in the popliteal artery than sedentary counterparts 2) aerobic training improves endothelial function in the brachial and popliteal arteries. Furthermore, there were no between groups differences in resting blood flow or peak blood flow in response to reactive hyperemia. Similarly, there were no between groups differences in resting conductance or peak conductance. Significantly more variability was demonstrated in trained subjects in comparison to sedentary subjects in the relationship between resting blood flow and FMD. In both groups, the brachial artery had more variability in the relationship between resting blood flow and FMD than the popliteal artery. This relationship was also seen both groups in the variability between peak blood flow and FMD.

It is known that risk factors for CVD begin in childhood and adolescence.[4] Therefore, it is essential to prevent the onset of disease in young adults with risk factor modification. Exercise has long been associated with good health, and it provides countless physiological

benefits. This study specifically investigated the changes in endothelial function associated with exercise training. Trained subjects were found via FMD assessment to have better endothelial function, making them less susceptible to atherosclerosis, a process that begins with damage to the endothelium.[15] Evaluating endothelial function via FMD is a cost effective and noninvasive test. Given the importance of preventative care and screening, it may be reasonable to utilize this technique in the clinic to assess patients' risk for CVD.[27] FMD may prove to be a valuable tool that saves health care dollars by identifying those at risk for CVD so that lifestyle modifications can be made before the disease progresses.

## Definitions

Myogenic responses- a change in blood flow to an organ due to smooth muscle responding to passive stretch or reduced stretch. Stretching of the artery results in smooth muscle vasoconstriction, and consequently a decrease in organ blood flow. Reduced stretch increases blood flow via vasodilatation, thus increasing blood flow to the organ.[15]

Humoral responses- a change in blood flow due to an excess of metabolically active tissues. An accumulation of nitric oxide, $H^+$, $K^+$, adenosine, and prostaglandins result in vasodilatation, and an increase in blood flow. Accumulation of endothelin, results in vasoconstriction and decreased blood flow.

Angiogenesis- growth of new blood vessels. An excess of growth may lead to diseases such as cancer and cardiovascular disease.[16]

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APPENDIX A: UMCRIB APPROVAL

East Carolina University

**EAST CAROLINA UNIVERSITY**
**University & Medical Center Institutional Review Board Office**
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Initial Approval (Committee)

From: Biomedical IRB
To: Anna Kate Bires
CC: Robert Hickner
Date: 12/10/2012
Re: UMCIRB 12-001801
Training and endothelial function in upper and lower limbs 1B

I am pleased to inform you that at the convened meeting on 11/28/2012 of the Biomedical IRB, the committee voted to approve the above study. Approval of the study and the consent form(s) is for the period of 11/28/2012 to 11/27/2013.

The Biomedical IRB deemed this study Greater than Minimal Risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The approval includes the following items:

| Name | Description | Modified | Version |
|---|---|---|---|
| Advertisement.doc \| History | Recruitment Documents/Scripts | 11/5/2012 8:25 AM | 0.03 |
| DEXA risk summary and questionnaire \| History | Surveys and Questionnaires | 11/19/2012 1:40 PM | 0.01 |
| Experimental Protocol Ex #2.doc \| History | Study Protocol or Grant Application | 11/30/2012 8:12 AM | 0.10 |
| Informed Consent - Ver 2.doc \| History | Consent Forms | 12/4/2012 5:12 PM | 0.06 |
| PAR-Q \| History | Surveys and Questionnaires | 11/16/2012 7:33 AM | 0.01 |
| Personal History - Short.doc \| History | Surveys and Questionnaires | 10/1/2012 11:23 AM | 0.02 |

APPENDIX B: INFORMED CONSENT

*East Carolina University*

**Consent to Participate in Research that is Greater than Minimal Risk**
**Information to Consider Before Taking Part in This Research**

Title of Research Study: Training and endothelial function in upper and lower limbs
Principal Investigator: Anna Kate Bires
Institution/Department or Division: ECU Human Performance Laboratory
Address:363 Ward Sports Medicine Building
Telephone #: (252) 737-4677

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Researchers at East Carolina University (ECU) study diseases, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find better ways to improve the lives of you and others. To do this, we need the help of people who are willing to take part in research.

You may have questions that this form does not answer. If you do have questions, feel free to ask the person explaining the study, as you go along. You may have questions later and you should ask those questions, as you think of them. There is no time limit for asking about this research.

You do not have to take part in this research. Take your time and think about the information that is provided. If you want, have a friend or family member go over this form with you before you decide. It is up to you. If you choose to be in the study, then you should sign the form when you are comfortable that you understand the information provided below. If you do not want to take part in the study, you should not sign this form. That decision is yours and it is okay to decide not to volunteer.

**Why is this research being done?**
The purpose of this research is to compare endothelial function (the health of the inner lining of your blood vessels) in the arm and leg in both trained (individuals who partake in at least thirty minutes of vigorous exercise three times per week) and untrained men (individuals who engage in less than one hour of vigorous exercise per week and who have not been involved in a structured exercise program in the past two months). Decreased endothelial function is a beginning step in cardiovascular disease. The decision to take part in this research is yours to make. By doing this research, we hope to learn how the endothelial function in both limbs of trained men compares to the endothelial function of untrained men.

**Why am I being invited to take part in this research?**
You are being invited to take part in this research because you are a healthy sedentary or trained male between the ages of 18 and 40. If you volunteer to take part in this research, you will be one of about 30 people to do so.

**Are there reasons I should not take part in this research?**
I understand that I should not volunteer for this study if I am under the age of 18 years; have a history of smoking; have a history of drug or alcohol abuse; have a history of liver, heart, or vascular disease; am unable to comply with the study requirements or provide valid informed consent; or have a condition that prevents me from doing normal daily activities. I understand that if I am considered sedentary, I do not exercise more than one day per week and have not participated in an exercise program in the past two months. I understand that if I am considered trained, I exercise at least thirty minutes a day three days a week.

**What other choices do I have if I do not take part in this research?**
You can choose not to participate.

**Where is the research going to take place and how long will it last?**
The research procedures will be conducted at the ECU Human Performance Laboratory. You will need to come to the HPL one time during the study. The visit will take about 2.5 hours. The total amount of time you will be asked to volunteer for this study is 2.5 hours over one visit scheduled at your convenience.

**What will I be asked to do?**
You are being asked to do the following: read and sign this Informed Consent for research; complete a Personal History Form to identify factors that would increase your risk of cardiovascular disease and to ensure that you are sedentary; complete a Physical Activity Readiness Questionnaire (PAR-Q) form prior to the exercise treadmill test - a "yes" answer to any question precludes participation in the study; undergo body composition analysis, a graded treadmill exercise test, and blood flow analysis of your leg and arm.

**Body Composition Measurement** – Height and weight will be recorded. Body composition will be assessed by a Dual-energy X-ray absorptiometry (DEXA) scan. A DEXA scan uses low doses of x-rays to scan the body and estimate fat mass, fat-free mass, and bone mass.

**Blood Flow Analysis** – Prior to your treadmill test we will measure the blood flow in your non-dominant leg and arm with ultrasound. This test, flow-mediated dilation (FMD), is a way to measure the function of the cells lining your arteries. You will lie on your back for 10 minutes with a pillow under your ankles while resting measurements are taken. Next, a blood pressure cuff will be inflated so that it feels very tight for 5 minutes. It will then be let down. You will be asked to lie still during the test. After the cuff is deflated, measurements will be made over a period of rest. This measurement will be repeated on your non-dominant arm.

**Graded maximal treadmill test** - You will be asked to complete a graded treadmill exercise test until exhaustion. The test follows the Bruce treadmill-based protocol. The Bruce protocol begins at a speed of 1.7 mph at a 10% grade. Every 3 minutes, the grade increases 2% and speed increases in increments between 0.5 to 0.8 mph. In order to measure the amount of oxygen and carbon dioxide you breathe, you will be fitted with a mouthpiece during the test. Oxygen consumption ($VO_2$), blood pressure, and heart rhythms will be monitored throughout the test and the test will be stopped promptly if you experience chest pain, dizziness, unusual shortness of breath, or if you request that the test be stopped.

**Bruce Treadmill Test Protocol**

| Stage | Time | Speed (mph) | Grade |
|---|---|---|---|
| 1 | 0:00-2:59 | 1.7 | 10% |
| 2 | 3:00-5:59 | 2.5 | 12% |
| 3 | 6:00-8:59 | 3.4 | 14% |
| 4 | 9:00-11:59 | 4.2 | 16% |

| | | | |
|---|---|---|---|
| 5 | 12:00-14:59 | 5.0 | 18% |
| 6 | 15:00-17:59 | 5.5 | 20% |
| 7 | 18:00-20:59 | 6.0 | 22% |
| 8 | 21:00-23:59 | 6.5 | 24% |
| 9 | 24:00-26:59 | 7.0 | 26% |
| 10 | 27:00-29:59 | 7.5 | 28% |

**What possible harms or discomforts might I experience if I take part in the research?**
There are always risks (the chance of harm) when taking part in research. We know about the following risks or discomforts you may experience if you choose to volunteer for this study. These are called side effects. The following side effects are known to occur in some people during exercise stress testing: dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death. These risks are very small, with an occurrence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. The risk is much smaller than this in healthy subjects. To minimize this risk, the PAR-Q will be administered to assess your readiness for exercise – a "yes" response to any question will preclude you from participating. Also, an Exercise Physiologist, who is trained to recognize heart problems during exercise and who is trained to revive people in the event of serious heart problems during the exercise testing will supervise the exercise stress test. The exercise stress test will be stopped if you feel dizzy, are having chest pain, are having serious shortness of breath, or ask that the test be ended. The exercise stress test will also be stopped if it is detected (from the ECG) that heart function is not normal. Additionally, there is a very small amount of radiation from the DEXA scan. The amount is less than would be received by being outside for a day and nearly 100 times less than the amount received from a chest x-ray.

**What are the possible benefits I may experience from taking part in this research?**
We do not know if you will get any benefits by taking part in this study. There may be no personal benefit from your participation, but the information gained by doing this research may help others in the future. You will have an exercise stress test, blood flow analysis, and body composition analysis performed.

**Will I be paid for taking part in this research?**
Yes, you will be compensated $25 for taking part in this research.

**What will it cost me to take part in this research?**
It will not cost you any money to be part of the research.

**Who will know that I took part in this research and learn personal information about me?**
To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- Any agency of the federal, state, or local government that regulates human research. This includes the Department of Health and Human Services (DHHS), the North Carolina Department of Health, and the Office for Human Research Protections.
- The University & Medical Center Institutional Review Board (UMCIRB) and its staff, who have responsibility for overseeing your welfare during this research, and other ECU staff who oversee this research.

**How will you keep the information you collect about me secure? How long will you keep it?**
Numeric coding will ensure the confidentiality of the volunteers for this study; the P.I. and study team members will have access to the code. Your collected data will be stored within a locked facility within the Human Performance

Laboratory. The data will be stored until successful reporting of this research has been completed. Data in paper format will be stored for a minimum of ten years and will be shredded prior to disposal. Electronic data will be eliminated from all storage devices after successful reporting of this research has been completed. All data will only be used in accordance with the informed consent agreement.

**What if I decide I do not want to continue in this research?**
If you decide you no longer want to be in this research after it has already started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive.

**Who should I contact if I have questions?**
The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at 724-372-3263.

If you have questions about your rights as someone taking part in research, you may call the Office for Human Research Integrity (OHRI) at phone number 252-744-2914 (days, 8:00 am-5:00 pm). If you would like to report a complaint or concern about this research study, you may call the Director of the OHRI, at 252-744-1971.

**Is there anything else I should know?**
No.

**I have decided I want to take part in this research. What should I do now?**
The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I know that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

**Participant's Name (PRINT)** **Signature** **Date**

**Person Obtaining Informed Consent**: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person's questions about the research.

**Person Obtaining Consent (PRINT)** **Signature** **Date**

APPENDIX C: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

## Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

| YES | NO | | |
|---|---|---|---|
| ☐ | ☐ | 1. | Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? |
| ☐ | ☐ | 2. | Do you feel pain in your chest when you do physical activity? |
| ☐ | ☐ | 3. | In the past month, have you had chest pain when you were not doing physical activity? |
| ☐ | ☐ | 4. | Do you lose your balance because of dizziness or do you ever lose consciousness? |
| ☐ | ☐ | 5. | Do you have a bone or joint problem that could be made worse by a change in your physical activity? |
| ☐ | ☐ | 6. | Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition? |
| ☐ | ☐ | 7. | Do you know of any other reason why you should not do physical activity? |

| If you answered: | YES to one or more questions |
|---|---|
| | Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.<br>• You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.<br>• Find out which community programs are safe and helpful for you. |

**NO to all questions**

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

**Delay becoming much more active:**

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: Reprinted from ACSM's Health/Fitness Facility Standards and Guidelines, 1997 by American College of Sports Medicine