THE EFFECT OF POSTURE AND FITNESS LEVEL ON POSTERIOR TIBIAL ARTERY SIZE

by

Kyle Shipp

(Under the Direction of Kevin McCully)

ABSTRACT

ARTERIAL DIAMETER has been shown to change in response to both acute and chronic stimuli. Exercise has been shown to result in a chronically elevated artery diameter, and chronic inactivity results in decreased artery size. Arterial range is a measure of the operating diameter in an artery, from minimum to maximum. Because it is unclear how arterial range changes as a result of exercise and posture, the purpose of this study was to examine the effect of supine and seated posture on arterial range in the posterior tibial artery in endurance-trained and untrained sedentary subjects. We examined arterial range in the posterior tibial artery in a supine and seated posture for each subject using Doppler ultrasound. We hypothesized that the seated posture would result in decreased arterial range in both endurance-trained and sedentary subjects, and that endurance training would increase arterial diameter and range. It was further hypothesized that an interaction between posture and endurance training would be present.

Arterial range, VO2peak, and fat-free mass from DXA were measured in 21 subjects. The mean VO2peak for the endurance-trained and untrained group was 65.37 ± 8.41 and 38.71 ± 8.00, respectively. Arterial diameter was similar between groups. Arterial range decreased by 21.5% from the supine to seated posture (p<0.01), but there was no significant effect of training status. In addition, the seated posture resulted in a 53.2% increase in resting arterial constriction.
(p<0.01), while there was no significant effect of training status. This was mainly due to an 8.1% increase in minimum diameter from the supine to seated posture (p<0.01).

Arterial range was significantly decreased in the seated versus supine posture. In addition, we were unable to detect a significant influence of chronic endurance training on sympathetic tone or structural remodeling in the posterior tibial artery.

INDEX WORDS: Arterial Diameter, Blood Flow Velocity, Arterial Range
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by

KYLE SHIPP

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KYLE SHIPP

Electronic Version Approved: Maureen Grasso
Dean of the Graduate School
University of Georgia
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DEDICATION

To my wife, Emily

Without your patience and sacrifice, I would not have made it this far.
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CHAPTER 1
INTRODUCTION

THE DIAMETER of arteries has been shown to change in response to both acute and chronic stimuli. Short-term increases in arterial size occur in response to increases in blood flow, mediated by increased nitric oxide bioavailability in the endothelium (33, 40). Exercise training has been shown to result in a chronically elevated artery diameter, and chronic inactivity results in decreased artery size (11, 17, 32) and reduced vascular reactivity to increases in blood flow (15). It is unclear if this is due to structural remodeling of the artery or altered sympathetic control (16).

Arterial lumen diameter and blood flow velocity can be measured with pulse-wave Doppler ultrasound imaging (26). Some researchers have used magnetic resonance spectroscopy imaging (MRI) to measure large artery size in clinical populations (3, 19, 30). Since the diameter of an artery is dynamic and reflects a multitude of acute and chronic factors, it is important to consider the physiological state of interest when reporting arterial diameter.

At rest in a supine position, blood flow is minimal, but sympathetic constriction of the vascular smooth muscle is also minimal. In an upright position, gravity increases the hydrostatic pressure experienced by the artery, and sympathetic tone increases to compensate. This will affect the diameter as well as the response to a blood flow challenge.

Flow-mediated dilation (FMD) is a non-invasive method of assessing peripheral arterial endothelial function (4). FMD is conducted by minimizing blood flow briefly (5 minutes) with a pneumatic cuff tourniquet, then releasing it rapidly to cause a large rush of blood into the artery
(reactive hyperemia). It reflects the ability of the smooth muscle of the endothelium to relax in response to shear stress induced by increased blood flow, and is dependent on the release of nitric oxide by the endothelium. The duration of occlusion for FMD is very important, as durations longer than 5 minutes can result in endothelium-independent stimuli for dilation, such as accumulation of metabolites and tissue oxygen desaturation. An occlusion period of approximately 10 minutes results in a maximal dilatory stimulus to the artery, which is independent of endothelial factors. We call this Maximal Dilatory Diameter (MDD).

A common clinical method of measuring maximal arterial diameter is nitroglycerin administration (41). A fixed sublingual dose results in systemic arterial dilation independent of changes in blood pressure or heart rate. The maximum arterial diameter obtained from nitroglycerin is similar to MDD, but no minimum diameter can be obtained.

STATEMENT OF THE PROBLEM

Currently, FMD and Arterial Range testing is performed in a supine position in both disabled and able-bodied clinical populations. However, humans perform most daily activities in a sitting or standing position. Differences in blood flow response due to posture changes have been observed in the upper and lower limbs at rest (21) and in response to ischemia (34). If sympathetic control in the arteries of the lower limb is different under the increased hydrostatic pressure of upright postures, then it would be a more realistic assessment of arterial function to perform FMD/MDD testing in an upright position such as sitting. Improving the methodology of non-invasive, in vivo tests of arterial function such as FMD/MDD is important for future research on clinical populations.
AIMS

Measure the size and range of the posterior tibial artery under two conditions (supine and seated) and in two groups of subjects (highly endurance trained and controls).

HYPOTHESES

1. Seated posture will reduce resting arterial diameter, maximal arterial diameter, and arterial range.
2. Highly endurance trained individuals will have larger resting diameters, and proportionally larger arterial range compared to individuals who do not perform regular endurance training.
3. There will be an interaction term between the effect of posture and endurance training on arterial range.

SIGNIFICANCE OF THE STUDY

Few studies have examined the effect of postural changes on arterial diameter or regulation in humans. The effects of endurance training on arterial diameter are also unclear. Because it is unclear how arterial range changes as a result of exercise, fitness, and posture, the purpose is to examine the effect of sitting versus standing on arterial range in the posterior tibial artery in highly-trained endurance athletes and in untrained sedentary subjects.
CHAPTER 2
REVIEW OF LITERATURE

VASCULAR REACTIVITY STUDIES

THE MAJORITY of existing literature on vascular reactivity has examined Flow-Mediated Dilation (FMD) in the brachial artery of the upper arm. Flow-Mediated Dilation has been used as a non-invasive evaluator of endothelial function (8) and as a marker of risk for cardiovascular disease (6). In the brachial artery, FMD is conducted by minimizing blood flow with a distal pneumatic tourniquet for 5 minutes, then measuring the blood flow response and arterial dilation that occurs after release of the cuff. FMD reflects the ability of the smooth muscle of the endothelium to relax in response to shear stress induced by increased blood flow, and is dependent on the release of nitric oxide by the endothelium. The duration of occlusion for FMD is very important, as durations longer than 5 minutes can result in endothelium-independent stimuli for dilation, such as accumulation of metabolites and tissue oxygen desaturation.

Although the majority of the literature relating endothelial dysfunction to cardiovascular disease focuses on the brachial artery as a model of endothelial function, vascular function has been assessed in more distal arteries that have a much different functional environment in terms of hydrostatic pressure and sympathetic tone, such as the radial, femoral, and posterior tibial arteries (1, 21, 23, 24).

Peripheral arteries in the legs experience a much greater level of hydrostatic pressure in an upright posture due to gravity, which must be overcome by a higher level of arterial
constriction (sympathetic tone). In addition, leg vasculature must cope with higher levels of chronic physical activity due to the role of the lower limbs in locomotion and posture.

The different endothelial environment of arteries in the legs versus the arms may lead to variation in arterial health within a single individual. Nishiyama et al. (19) examined FMD and ischemic reperfusion following 5 minutes of cuff ischemia in the brachial and popliteal arteries of healthy young (n=12) and healthy old (n=12) subjects using ultrasound. The brachial artery testing occurred in a supine position with a pneumatic cuff placed distal to the ultrasound probe and proximal to the elbow. The cuff was inflated to >250 mmHg for 5 minutes, following a 20-minute rest period and baseline measurements of arterial diameter, blood flow velocity and intima-medial thickness. The popliteal artery testing occurred in a supine posture on a gurney modified to allow dorsal access to the popliteal artery. The pneumatic cuff was again placed distal to the artery and inflated to suprasystolic pressure (>250 mmHg) following a 20-minute resting period. Total blood flow and shear rate were calculated before and after ischemia. FMD was significantly attenuated in both arteries in the old group (Brachial artery 0.028 ± 0.002 cm young group, 0.018 ± 0.002 cm old group; Popliteal artery 0.031± 0.003 young group, 0.009 ± 0.003 old group). There was a greater attenuation in the popliteal artery than in the brachial artery of the old group. Additionally, the investigators reported significant age-related attenuation of shear rate Area under the Curve in both the brachial artery and popliteal artery, and between arteries in both groups. When FMD was normalized for shear rate, the old group showed a preserved FMD relative to the young group in the BA but not the PA (p<.05). The investigators suggested that endothelial function in the legs is altered by aging in a different manner than the arms, and that the difference in FMD cannot entirely be explained by differences in shear stimulus.
EXERCISE, DETRAINING, AND VASCULAR REMODELING

Chronic exercise results in extensive vascular adaptations in skeletal muscle, including remodeling of arteries, increase in the density of arterioles, and capillary angiogenesis (17). Exercised-induced vascular remodeling (capillary angiogenesis, increase in arteriolar density) has been well-documented in both human (7, 10), and animal models (4, 15).

Exercise training increases blood flow capacity of muscle by altered vascular control and vascular remodeling (27). Walther et al. measured post-occlusion FMD, glycerine trinitrate-induced dilation, and exercise-induced blood flow alterations in the brachial and superficial femoral arteries of 21 athletes (11 cyclists, 10 swimmers), and 10 sedentary controls. The athletes had been nationally competitive for at least 5 years. All subjects were tested for VO$_2$max and limb muscle mass (from DXA) prior to the vascular testing sessions. For the FMD measurements, the occlusion cuff was placed distal to the ultrasound Doppler probe and inflated for 5 minutes. Glycerine trinitrate-induced dilation was measured after administration of 0.4 mg glycerine trinitrate. Finally limb blood flow and muscle perfusion was measured in the upper and lower limbs during progressively harder elbow flexion or knee extension in the supine position until exhaustion. Both groups of athletes showed a greater FMD post-occlusion than the sedentary group. Swimmers showed a significantly greater FMD in the brachial artery than cyclists, while this was reversed in the superficial femoral artery. This increase in FMD in athletes was still present after normalizing for shear rate area under the curve, indicating that increased endothelial function (greater bioavailability or increased sensitivity to nitric oxide), is a persistent adaptation to exercise. A similar outcome was observed for glycerol trinitrate-induced dilation. Swimmers demonstrated significantly greater peak arterial diameter than cyclists or sedentary individuals in the brachial artery, while cyclists demonstrated significantly greater
peak diameter in the superficial femoral artery than swimmers or sedentary individuals. Muscle perfusion and vascular conductance followed the same pattern as glycerol trinitrate-induced dilation. Swimmers exhibited 15-67% and 8-33% higher muscle perfusion and vascular conductance than sedentary individuals and cyclists, respectively during the elbow flexion test. Cyclists exhibited 12-79% and 11-44% greater perfusion and conductance than sedentary individuals and swimmers during the knee extension test. The researchers concluded that changes in arterial control and blood flow capacity associated with exercise occur in a limb-specific manner.

**ARTERIAL HEALTH AND STIFFNESS**

Arterial stiffness is a general term that signifies a reduction in compliance, which is the change in arterial diameter for a given change in intravascular pressure. Increased arterial stiffness is associated with increased risk of cardiovascular and renal disease (13).

An artery with increased arterial stiffness will have a reduced variation between systolic and diastolic diameter. Vaitkevicius et al. (25) calculated the arterial stiffness index using common carotid arterial pressure pulse augmentation index (AGI) and Doppler ultrasound pulse-wave velocity in the common carotid, ascending aorta, and right femoral artery in 146 healthy, normotensive individuals of both sexes ranging from 20 to 91 years, including 14 distance runners aged 54 and greater. Pulse augmentation index is determined using a pulse-pressure waveform obtained by applanation tonometry, in which a pencil-like probe containing a Millar manometer is placed over a superficial artery such as the carotid. The pressure waveform of the artery across the cardiac cycle is thus obtained.

Pulse augmentation index is determined by the difference in pressure between the “foot” of the waveform immediately prior to systole, and the peak pressure at systole. As arterial
stiffness increases, diastolic flow makes less of a contribution to forward flow in the artery, lowering the arterial pressure at the beginning of systole, and thereby increasing the difference in pressure. The investigators then determined VO$_2$max with a treadmill maximal exercise test for each subject. The investigators reported that over the age range examined, arterial pulse-wave velocity doubled, and pulse augmentation index increased fivefold, despite a mere 14% increase in systolic blood pressure within the normal range. Each index of arterial stiffness (systolic pressure, pulse-wave velocity, and AGI) was significantly and inversely related to VO$_2$max. A multiple regression model revealed a marked effect of VO$_2$max on pulse augmentation index independent of age, and an effect of age on AGI independent of sex, BMI, and VO$_2$max.

The effect of endurance training on AGI was examined by comparing the senior male endurance athletes with age-matched and young male controls. The senior athletes had a 25.9% lower pulse-wave velocity than the age-matched controls, and were not significantly different from the young controls. AGI was significantly greater in the age-matched controls than in the senior athletes, and the senior athletes had a significantly higher AGI than the young controls. The investigators concluded that arterial stiffness was inversely related to chronic exercise habits, and that the age-related increase in arterial stiffness could be mitigated by aerobic exercise in normotensive adults of both sexes.

**ROLE OF THE ENDOTHELIUM IN CARDIOVASCULAR DISEASE**

The endothelium is a single layer of cells lining the inner surface of the entire vascular system. The endothelium has autocrine, paracrine, and endocrine functions, and plays a role in activities such as vasoreactivity to changes in blood flow, immune response, platelet aggregation, and thrombolysis (26). Endothelial dysfunction is known to be a risk factor for cardiovascular disease (2). Endothelial function can be assessed non-invasively with flow-mediated dilation.
Atkov et al. (3) examined brachial artery FMD and nitroglycerin-induced dilation with ultrasound in three groups of subjects: 12 subjects with essential hypertension, 10 subjects with familial hypercholesterolemia, and 10 controls with no vascular risk factors. The investigators reported a significant attenuation of FMD along with a significantly greater reactive hyperemia in the hypercholesterolemia group, but not the hypertension group. Nitroglycerin-induced dilation was reduced in the hypercholesterolemia and hypertension groups, but this was not statistically significant. The investigators concluded that endothelial health could be successfully evaluated using FMD and ultrasound. They noted that differences in medication among the subjects with hypertension may have been a confounding variable that prevented a significant impairment of FMD from being detected in this group, as had been reported elsewhere in the literature(12).

MEASURING ARTERIAL RANGE

Olive et al. (20) in 2002 examined the effect of various cuff-ischemia durations (2, 4 and 10 minutes of ischemia) on blood flow response in the femoral artery in incomplete SCI-injured subjects versus able-bodied controls. Five incomplete SCI’s and seventeen able-bodied controls underwent three trials of cuff ischemia of various durations (2, 4, and 10 minutes) in random order. The investigators reported that a 10-minute distal cuff ischemia-release protocol resulted in a maximal peak blood-flow response after release of the cuff, compared to 2 and 4-minute trials. The magnitude of the blood flow response is the main factor in the magnitude of the dilatory shear-stress stimulus.

Naylor et al. (18) examined the effect of various combinations of distal ischemia, ischemic exercise, and sublingual nitroglycerin (GTN) on arterial range in the brachial artery of healthy adults (33% female, mean age 36.7 (9.2) years). Each subject underwent nine testing
sessions, in randomized order: cuff ischemia of 5, 10, and 15 minute durations, GTN administration, GTN administration and cuff ischemia of 5, 10, and 15 minutes, 5 minute cuff ischemia including 3 minutes isotonic handgrip exercise, and 5 minute cuff ischemia including 3 minutes isotonic handgrip in addition to GTN administration. Peak arterial diameter was observed in response to GTN, 5 minute ischemia, and handgrip exercise. The effect of adding GTN to ischemic and ischemic exercise protocols was not statistically significant, but the addition of exercise to the ischemic protocols resulted in a statistically significant increase in maximum arterial diameter. In the cuff ischemia trials, the magnitude of arterial dilation was non-significantly, positively related to the duration of the cuff ischemia, with a much smaller difference between 10 and 15 minutes.

Stoner et al. (23) examined FMD and arterial range in subjects with complete Spinal Cord Injury (SCI) in the radial and posterior tibial arteries. Subjects were tested in the supine position, and underwent 5 minute cuff ischemia, followed by 10 minute cuff ischemia for evaluation of FMD and arterial range respectively. The investigators reported impaired arterial range (maximum-minimum) in the posterior tibial artery in complete SCI versus normal controls, but this was due to a greater minimum arterial diameter (7% greater in SCI), and not a reduction in the maximum diameter.

**SYMPATHETIC CONTROL OF ARTERIAL DIAMETER**

Sabatier et al. (21) examined the effect of posture on arterial range in the posterior tibial artery in healthy subjects. Eight subjects (4 male, 4 female) were evaluated for resting posterior arterial diameter by different observers, and on different days, to evaluate day-to-day and inter-observer reliability. Afterwards, eight male subjects were then tested for postural differences in posterior tibial arterial range during a 10-minute proximal cuff ischemia protocol. Each subject
was tested in a supine and sitting posture, with 3-6 days separating testing sessions. The sitting posture resulted in and decreased resting diameter over the supine position (2.4 ± 0.3 mm vs. 2.6 ± 0.2 mm), as well as an increase in minimum (2.4 ± 0.2 vs. 2.1 ± 0.2 mm) diameter during ischemia. After release of the cuff, maximum arterial diameter was decreased in the sitting posture versus the supine posture (2.8 ± 0.3 vs. 3.1 ± 0.4 mm). This supports the hypothesis that increased sympathetic tone exists in upright postures to counter increases in hydrostatic pressure due to gravity. The investigators suggested that the larger minimum diameter observed in the sitting posture was due to this increase in hydrostatic pressure.

**ENDURANCE ATHLETES**

Chronic endurance training has been shown to increase the diameter of central arteries such as the carotid artery and abdominal aorta as well as peripheral arteries such as the femoral artery (9, 11). Huonter et al. measured the diameter and blood flow velocity of the thoracic and abdominal aorta, the subclavian and common femoral artery using duplex sonography in 18 professional tennis players, 34 elite road cyclists, 26 athletes with paraplegia, 17 athletes with below-knee amputations, and 30 able-bodied, untrained subjects. The investigators reported a 21% greater diastolic lumen diameter in the femoral artery of the cyclists versus the tennis players and controls, whereas the tennis players had a 19% larger diastolic lumen diameter in the subclavian artery of the racquet arm versus the other arm. No differences were observed between groups in the thoracic or abdominal aorta. The investigators asserted that the use of diastolic diameter was more reflective of possible structural changes in the artery wall itself, rather than increased dilation due to a decrease in sympathetic tone or local vasodilatory factors.
SIGNIFICANCE TO DISEASE POPULATIONS

Populations with spinal cord injury (SCI) have a 228% higher incidence of mortality due to cardiovascular disease that the general population (14). The leading causes of death in the SCI population are septicemia, cancer, and heart disease (22). There is a clear need for greater understanding of the pathogenesis of cardiovascular disease after SCI, particularly in the vasculature in limbs affected by paralysis. In other populations such as chronic fatigue syndrome, multiple sclerosis, metabolic syndrome or incomplete paralysis, evaluation of arterial and endothelial function in peripheral arteries in the lower limbs may provide a more complete picture of arterial health than upper-limb testing (5), since the legs are most often targeted by exercise interventions and experience a higher level of chronic physical activity. Black et al. (5) assessed the feasibility of conducting FMD testing in the posterior tibial artery of healthy able-bodied subjects. The investigators reported that day-to-day variability within subjects was comparable to variability reported in the literature for the traditionally-used brachial artery. Additionally, the investigators used a novel method of comparing FMD with arterial constriction ((max-baseline)/(max-minimum)*100%) to compare the relative degree of hyperemia between subjects with varying arterial diameters. An $R^2$ value of 0.73 was reported for the correlation between FMD and vascular tone in this study.
REFERENCES


CHAPTER 3

THE EFFECT OF POSTURE AND FITNESS LEVEL ON POSTERIOR TIBIAL ARTERY SIZE

ABSTRACT

K. Shipp, T. Nguyen, L. Nielsen, and K. McCully. The effect of posture and fitness level on posterior artery size. Arterial diameter has been shown to change in response to acute and chronic stimuli. Exercise elevates artery diameter, and chronic inactivity decreases it. Arterial range is a measure of the range of diameter in an artery (maximum-minimum). Because it is unclear how arterial range changes as a result of exercise and posture, the purpose of this study was to examine the effect of supine and seated posture on arterial range in the posterior tibial artery in endurance-trained and untrained subjects. We examined arterial range in the posterior tibial artery in a supine and seated posture using ultrasound.

Arterial range, VO\textsubscript{2}peak, and DXA fat-free mass were measured in 21 subjects. The mean VO\textsubscript{2}peak for the endurance-trained and untrained group was 65.4 ± 8.4 and 38.7 ± 8.0, respectively. Arterial diameter was similar between groups, although the untrained group had a significantly greater minimum diameter in the seated posture. Changing from the supine to seated posture resulted in a 21.5% decrease in arterial range (p<0.01) but there was no significant effect of training. Changing from the supine to the seated posture resulted in an overall 53.2% increase in resting arterial constriction (p<0.01), with no significant effect of training status. This was due to an 8.1% increase in minimum diameter from the supine to seated posture (p<0.01). We conclude that arterial range is greater in the supine than seated posture. In addition, we were unable to detect a significant influence of endurance training on sympathetic tone or remodeling in the posterior tibial artery.

Keywords: Arterial Diameter; Blood Flow Velocity; Arterial Range
INTRODUCTION

ARTERIAL DIAMETER is not constant, but has been shown to change in response to acute and chronic stimuli. Changes in arterial diameter can result from changes in blood flow, mediated by levels of nitric oxide bioavailability in the endothelium, as well as a variety of other factors (14, 17). Exercise training has been shown to chronically elevate arterial diameter, while chronic inactivity results in decreased artery size (8, 13) and reduced vascular reactivity to increases in blood flow (6). The degree to which this is due to structural remodeling of the artery or altered sympathetic control (7) is unclear.

Arterial lumen diameter and blood flow velocity can be simply and non-invasively measured with Doppler pulse-wave ultrasound imaging (11). Since the diameter of an artery is dynamic and reflects a multitude of acute and chronic factors, it is important to consider the physiological state of interest when reporting arterial diameter. For example, arterial function may be influenced by changes in hydrostatic pressure associated with postural shift. In an upright posture, gravity increases the hydrostatic pressure experienced by the artery, and sympathetic tone may increase to compensate. This will affect the diameter as well as the response to a blood flow challenge.

Flow-mediated dilation (FMD) is a non-invasive clinical method of assessing peripheral arterial endothelial function (1), specifically the sensitivity of the endothelium to nitric oxide bioavailability. FMD is measured by minimizing blood flow in a limb briefly (5 minutes) with a pneumatic cuff, then releasing it, resulting in a large rush of blood into the artery (reactive hyperemia). The duration of occlusion for FMD is very important, as durations longer than 5 minutes can result in endothelium-independent stimuli for dilation, such as accumulation of metabolites and tissue oxygen desaturation. An occlusion period of approximately 10 minutes
results in a maximal dilatory stimulus to the artery, which is independent of endothelial factors. We refer to the resulting dilation as Maximal Dilatory Diameter. By subtracting the minimum diameter measured during cuff occlusion from the maximum diameter post-hyperemia, we generate a measure called Arterial Range that encompasses the operating range of the artery.

Another clinical method of measuring maximal arterial diameter is nitroglycerin administration (18). A fixed sublingual dose results in systemic arterial dilation independent of changes in blood pressure or heart rate.

Arterial Range testing is typically performed in a supine posture. However, humans perform most daily activities in a sitting or standing position. Differences in blood flow due to posture have been observed in the upper and lower limbs at rest (9) and in response to ischemia (15). If sympathetic control in the arteries of the lower limb is different under the increased hydrostatic pressure of upright postures, then the arterial range measurement could be affected. The purpose of this study was to measure the size and arterial range of the posterior tibial artery in supine and seated postures in endurance-trained and sedentary subjects.

It is hypothesized that the seated posture results in a decreased arterial range in both endurance-trained and sedentary subjects, and that endurance training increases arterial diameter and range. It is further hypothesized that an interaction between the effect of posture and endurance training on arterial range exists, and that chronic endurance training may reduce sympathetic tone, resulting in less arterial constriction in the seated posture.
METHODS

Subjects

The study was conducted with the approval of the institutional review board at the University of Georgia. All subjects provided written informed consent before undergoing tests. Subjects were young, healthy adults between 18 and 30 years of age with no contraindications for maximal exercise.

Subjects classified as endurance-trained spent approximately 15 hours per week engaged in strenuous endurance exercise, and had a VO$_2$peak of >65 ml/kg/min (males) or >55 ml/kg/min (females). Sedentary subjects spent no more than 1 day per week engaged in strenuous exercise and had a VO$_2$peak of <45 ml/kg/min (males) or <40 ml/kg/min (females).

Volunteers with a history of cardiovascular and/or neuromuscular disease, evidence or history of other significant metabolic disease, untreated hypertension, body mass index (BMI) greater than 32, anemia, polycythemia or sickle cell anemia, blood clotting disorders, a habit of smoking, or who were taking oral contraceptives or other vasoactive medications were excluded from the study.

Each subject was briefly interviewed prior to the first testing session to ensure that none of these contraindications were present.

Experimental Design

Testing was conducted in two sessions of approximately 1.5 hours each. The first session consisted of reading and signing the informed consent agreement, completing a habitual physical activity questionnaire (IPAQ Short Form), and exercise safety screening. The IPAQ describes vigorous physical activity as running, fast cycling, heavy weightlifting, etc, and moderate activity as doubles tennis, hiking, moderate cycling, etc (4).
Experimental Procedures

The subjects then underwent a VO$_2$peak test. The second session took place no sooner than 24 hours after the first, and consisted of the vascular ultrasound test described below and DXA scan. Subjects were asked to abstain from caffeine, exercise, alcohol, drugs and high-fat meals on the days of testing. The VO$_2$peak test was conducted on a Lode Excalibur electronically-braked cycle ergometer, using a ParvoMedics TrueMax 2400 metabolic cart (ParvoMedics, Salt Lake city, Utah). Each subject was given a 5-minute familiarization trial of cycling at a low intensity to become accustomed to breathing through the apparatus prior to starting the test. The test began with 1 minutes of pedaling at a workload of 150 Watts, at self-selected cadence. Then the workload increased by 1 Watt every 2 seconds until the subject was unable to maintain at least 50 RPM at maximal effort. After exhaustion, the subject pedaled at 50 Watts for 3 minutes to cool down. Immediately following the test, the subject indicated terminal RPE on a 0-20 scale.

All testing was conducted between 8:30 AM and 3:00 PM. The vascular ultrasound test began with 10 minutes of rest in the supine posture to allow equilibration of heart rate and blood flow to a resting state. During this period, a pneumatic cuff attached to a Hokannsen rapid pressure inflater was fitted to the thigh, just above the knee. Blood pressure and heart rate were monitored during this period with a Datascope Accutorr 3 automated blood pressure cuff. After the equilibration period, blood flow and arterial diameter were measured in the brachial and femoral artery proximal to the bifurcation into the circumflex femoral artery.

Blood flow velocity and arterial diameter were recorded in the posterior tibial artery at rest, and then the occlusion cuff was inflated to a pressure of systolic BP +100 mmHg for 10 minutes. At minute 2 of ischemia, arterial diameter was recorded to obtain minimum diameter.
Blood flow velocity was measured for 15 seconds post-release, and arterial diameter was recorded continuously for a 2 minute recovery period to evaluate peak arterial diameter.

The subjects were then moved to a seated position, and waited for a further 10 minute equilibration period. The above protocol was repeated as before in the sitting posture. Each subject was then given a DXA scan following the ultrasound test to normalize arterial size to fat-free mass. Whole-body fat-free mass was used to normalize arterial diameter by dividing the diameter in millimeters by fat-free mass in kilograms. Lean mass of each thigh and lower leg was also measured by analyzing the area inside a box drawn from a line bisecting the knee at the femur/tibia junction up to a horizontal line across the thigh at the distal base of the perineum. The lower leg lean mass was measured by analyzing the volume inside a box drawn bisecting the knee at the femur/tibia junction down to a line bisecting the ankle across the distal tip of the medial and lateral malleolus of the tibia and fibula.

Data Analysis

The 16-second videos recording were saved on the ultrasound hard drive, optimized for image quality, and then transferred to a mass-storage hard drive. Each video clip was then decompiled into individual jpeg images and analyzed with custom software. Arterial diameter was determined from B-mode images alone. Diameter was reported as the mean diameter across three consecutive cardiac cycles per clip. Blood flow velocity was measured over a 16-second time frame in pulse-wave Doppler mode, using a custom-written program to measure mean and maximum blood velocity across the cardiac cycle. Velocity was reported as the time-averaged mean ± SD velocity across several cardiac cycles. A small pilot study found 4.1% variability on repeat measures of arterial diameter.
**Statistical Analysis**

Hypothesis testing was performed in SPSS 17.0 using a mixed-model Analysis of Variance (ANOVA). Each hypothesis was tested individually, and was to be accepted if a significant ($p<0.05$) interaction was found. If no interaction was present, testing for main effects was performed.

**RESULTS**

Table 3.1 shows the physical characteristics of the subjects. There was a significant difference between groups for VO$_2$peak ($p<0.01$), diastolic blood pressure ($p<0.05$), body fat percentage ($p<0.01$) and training volume ($p<0.01$). The endurance-trained group performed $386 \pm 200$ min/week of vigorous exercise ($\approx 8$ METs), and $399 \pm 209$ min of moderate activity ($\approx 4$ METs). The untrained group performed $50 \pm 61$ min/week of vigorous activity and $57 \pm 46$ min/week of moderate activity.

**VO$_2$peak**

VO$_2$peak values for each group are presented in Table 3.1. The endurance-trained group exhibited a peak oxygen uptake of $65.4 \pm 8.4$ ml/kg/min, while the untrained group peaked at $38.7 \pm 8.0$ ml/kg/min. Each subject reported at least 17 on the 0-20 RPE scale after termination of the test. Peak RER values were not significantly different between groups. The endurance-trained group exhibited a peak RER of $1.2 \pm 0.06$, while the untrained group exhibited a peak RER of $1.22 \pm 0.07$ ($p>0.05$).

**Arterial Diameter**

Arterial diameter results are presented in Table 3.2 to 3.4. Arterial diameter is given as (diameter/ fat-free mass) to normalize for the large differences in arterial diameter and body mass between subjects. No significant interaction was found for posture and fitness level for resting diameter ($F=0.119$), minimum diameter ($F=1.385$), maximum diameter ($F=0.413$) or
arterial range (F=3.014). Additionally, no significant interaction for posture and fitness level was found for arterial constriction (F=0.876), resting velocity (F=0.106) or peak hyperemic velocity (F=0.030). Since no significant interactions were found, testing for main effects was performed.

Figure 3.1 shows a representative B-mode image of the posterior tibial artery in the seated posture at rest. The artery reached the minimum observed diameter at approximately 2-3 minutes after the onset of cuff ischemia, and then slowly dilated in both postures until release of the cuff. After the release of the cuff, peak arterial diameter was observed at 60-90 seconds in the supine posture and 45-60 seconds in the seated posture. There were no significant differences between postures for baseline (p=0.15) and maximum diameter (p=0.41), but minimum diameter was significantly greater in the seated posture (p<0.01). Interestingly, the minimum diameter was significantly larger in the sedentary group (p<0.01). This could be due to slightly but not significantly lower FFM in the sedentary group (p>0.05). No significant differences were found between groups in the brachial and femoral arteries at rest (Fig. 3.5). There was a non-significant trend towards a sex difference in arterial diameter observed in the present study. Females exhibited a 9% greater baseline diameter than males in both postures and a 10% greater minimum diameter (p>0.05).

Blood Flow Velocities

Table 3.3 presents pulse-wave velocity data. There was a non-significant (p=0.07) trend toward higher resting blood flow velocity in the brachial artery in the sedentary group, and blood flow velocity in the femoral artery was 38% higher (p<0.05) in the sedentary group. There were no group differences for blood flow velocity in the posterior tibial artery. Seated baseline velocity was 42% lower than supine velocity, and seated maximum velocity was 7% lower than supine, though this was not significant (p>0.05).
**Arterial Range**

Arterial range (maximum-minimum) data is included in Table 3.2. There were no significant differences in arterial range between sedentary and endurance-trained individuals. Overall, arterial range decreased by 21% from the supine to seated posture (p<0.01). This was mainly due to the increased minimum diameter in the seated posture.

However, there was a non-significant (p>0.05) increase in arterial range in the untrained group from supine to seated. This resulted in a nearly-significant trend (p=0.053) towards a difference in arterial range between groups. Female subjects exhibited a 14% trend towards greater overall arterial range than males, though it did not reach statistical significance (p>0.05).

**Constriction**

Arterial constriction ((max-baseline)/range *100%) is given in Table 3.2. This may represent the degree of sympathetic tone affecting an artery. An artery with less sympathetic tone would exhibit less constriction. Constriction increased by 53% from the supine to seated posture (p<0.01), but there were no significant differences in constriction between groups for either posture.

**DISCUSSION**

**Agreement with Previous Literature**

Sabatier et al. (15) performed a very similar experiment as the current study in eight recreationally-active males of college age. The authors reported that arterial range in the seated posture decreased by approximately 44% compared to supine, whereas in the current study we observed a decrease of 22% in the seated posture compared to supine. This may be due to the fact that female subjects, regardless of training status, exhibited a non-significant (p>0.05) trend towards greater arterial range in the seated posture than males. This trend was mainly due to the
untrained female subjects displaying greater arterial range in the seated posture versus supine, while arterial range decreased when moving to the seated posture in all other groups.

Previous research has shown that endurance-trained athletes typically exhibit a greater arterial diameter at rest than untrained but healthy subjects in trained limbs (2, 16). This has been postulated to be due to a combination of increased resting dilation and structural remodeling of the artery wall (12). In the present study, no significant difference in brachial or femoral resting diameter was observed between groups in either absolute diameters or after normalizing the arterial diameter for whole-body fat-free mass. The arterial diameter data was reanalyzed in SPSS with an ANCOVA using thigh lean mass (fat-free mass minus bone mineral mass) as a covariate, but this did not impact the statistical significance. This may be partially due to the non-significant trend towards a larger normalized arterial diameter in females, who made up a greater proportion of subjects in the untrained group.

Sex Differences in Autonomic Function

Convertino (3) reported a lower tolerance to an orthostatic challenge in females versus males. This difference was attributed to, among other causes, a greater vasoconstriction for a given level of orthostatic challenge. Frey et al. (5) similarly reported a greater increase in calf circumference and peripheral vascular resistance in men versus women, and Jarvis et al. (10) reported an attenuated splanchnic vasoconstrictor response to a 70-degree head-up tilt in women versus men, which contributed to a trend towards decreased tolerance of the orthostatic challenge.

In the case of supine versus seated arterial diameter measurements, as in the present study, a lower response to the orthostatic challenge in women would be expected to manifest itself as a larger baseline and minimum diameter than men. Indeed, when the data is examined
for a difference due to sex rather than training level, there is a non-significant trend toward a 9% greater baseline diameter (p=0.06) and a 10% greater minimum diameter (p=0.07) in women.

Limitations

There was an imbalance between males and females between groups. Although there were not enough subjects to evaluate it statistically, sex may have resulted in a greater difference in arterial diameter than endurance training in this study. Future research may be necessary to evaluate the effect of sex on arterial regulation. Although all subjects were asked to refrain from exercise on the day of testing, it is possible that there may have been lingering acute effects of vigorous exercise from the previous day. The possible acute effects and time course of exercise on measurement of arterial range have not been investigated.

This study was somewhat underpowered to detect some differences that did not quite reach statistical significance. If the total sample size was closer to 20 subjects per group, rather than 10, the conclusions may have been different.

Conclusion

Arterial range decreased significantly in the seated posture versus the supine posture in the posterior tibial artery in both endurance-trained and untrained groups. There was no difference in minimum or maximum diameter between groups, although there was a non-significant trend towards greater seated arterial constriction in the untrained group. Potential sex differences in autonomic function might have obscured training-induced changes in arterial regulation, but further research is needed to clarify the impact of sex on arterial regulation.
ACKNOWLEDGEMENTS

The authors express their gratitude to the graduate and undergraduate students of the Kinesiology Department at UGA for volunteering as technical assistants and research subjects.
REFERENCES


Table 3.1 Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Athletes (n=11)</th>
<th>Nonathletes (n=10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 ± 7</td>
<td>175 ± 14</td>
<td>p=0.95</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.7 ± 7.18</td>
<td>70.0 ± 13.9</td>
<td>p=0.31</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>51.9 ± 5.81</td>
<td>50.4 ± 13.8</td>
<td>p=0.74</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>19.5 ± 6.4</td>
<td>28.6 ± 7.7</td>
<td>p=0.004*</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>21.1 ± 2.2</td>
<td>22.8 ± 3.3</td>
<td>p=0.09</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 ± 2</td>
<td>21 ± 3</td>
<td>p=0.26</td>
</tr>
<tr>
<td>VO$_2$ peak (ml/kg/min)*</td>
<td>65.37 ± 8.41</td>
<td>38.71 ± 8.00</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>VO$_2$ peak (ml/kgFFM/min)*</td>
<td>80.99 ± 6.63</td>
<td>54.02 ± 7.78</td>
<td>p&lt;0.001*</td>
</tr>
<tr>
<td>Training (hrs/week)*</td>
<td>14 ± 4</td>
<td>2 ± 1</td>
<td>p&lt;0.001*</td>
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<tr>
<td>Resting Heart Rate (bpm)</td>
<td>60 ± 9</td>
<td>64 ± 11</td>
<td>p=0.28</td>
</tr>
<tr>
<td>Blood Pressure (mmHg)*</td>
<td>117/62</td>
<td>113/68</td>
<td>p=0.04*</td>
</tr>
</tbody>
</table>
### Table 3.2 Arterial Diameter Results: Posture

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Supine</th>
<th>Seated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (mm/kgFFM)</td>
<td>0.044±0.008</td>
<td>0.043±0.008</td>
<td>P=0.15</td>
</tr>
<tr>
<td>Minimum (mm/kgFFM)</td>
<td>0.037±0.007</td>
<td>0.040±0.008</td>
<td>P=0.005*</td>
</tr>
<tr>
<td>Maximum (mm/kgFFM)</td>
<td>0.052±0.008</td>
<td>0.052±0.008</td>
<td>P=0.41</td>
</tr>
<tr>
<td>Range (max-min)</td>
<td>0.014±0.004</td>
<td>0.012±0.004</td>
<td>P=0.005*</td>
</tr>
<tr>
<td>Constriction ([max-baseline]/range]*100%)</td>
<td>50.17±19.93%</td>
<td>76.89±17.59%</td>
<td>P=0.001*</td>
</tr>
</tbody>
</table>
Table 3.3 Arterial Diameter Results: Group

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Endurance-trained</th>
<th>Untrained</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (mm/kgFFM)</td>
<td>0.042±0.007</td>
<td>0.044±0.008</td>
<td>P=0.45</td>
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<tr>
<td>Minimum (mm/kgFFM)</td>
<td>0.037±0.007</td>
<td>0.037±0.008</td>
<td>P=0.98</td>
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<tr>
<td>Maximum (mm/kgFFM)</td>
<td>0.050±0.007</td>
<td>0.053±0.008</td>
<td>P=0.23</td>
</tr>
<tr>
<td>Range (max-min)</td>
<td>0.013±0.005</td>
<td>0.017±0.008</td>
<td>P=0.053</td>
</tr>
<tr>
<td>Constriction ((max-baseline)/range)*100%</td>
<td>60.63±25.45%</td>
<td>66.72±20.06%</td>
<td>P=0.20</td>
</tr>
</tbody>
</table>
**Table 3.4 Blood Flow Velocity**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Condition</th>
<th>Value</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Brachial Artery (cm/s)</td>
<td>Endurance-trained</td>
<td>9.25±4.75</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>12.1 ±4.89</td>
<td></td>
</tr>
<tr>
<td>Femoral Artery (cm/s)</td>
<td>Endurance-trained</td>
<td>8.12±3.52</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>11.23±4.66</td>
<td></td>
</tr>
<tr>
<td>Posterior Tibial</td>
<td>Supine</td>
<td>3.00±2.35</td>
<td>0.07</td>
</tr>
<tr>
<td>Baseline (cm/s)</td>
<td>Seated</td>
<td>2.11±1.27</td>
<td></td>
</tr>
<tr>
<td>Posterior Tibial</td>
<td>Supine</td>
<td>20.13±6.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Maximum Hyperemic (cm/s)</td>
<td>Seated</td>
<td>18.78±7.51</td>
<td></td>
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</tbody>
</table>
FIGURE LEGENDS

Fig 3.1: Representative B-mode image of posterior tibial artery in seated posture in an endurance-trained female.

Fig 3.2: Representative Excel histogram plot of posterior tibial arterial diameter data. Diameter is recorded three times per second, and diameter data is reported as mean ± SD of three consecutive cardiac cycles. Outlier data points were excluded from analysis.

Fig 3.3: Representative PW-mode image of posterior tibial artery blood flow velocity in seated posture. Live B-mode image is seen at the top, while the velocity waveform is recorded on the bottom of the image.

Fig 3.4: Representative Excel histogram plot of posterior tibial artery blood flow velocity data. Blood flow velocity is reported as the TAMEAN in cm/s across three consecutive cardiac cycles.

Fig 3.5: Brachial artery diameter (mm/kgFFM) in males (black) and females (white). Mean±SD values were 0.07 ± 0.008 and 0.07 ± 0.01 mm/kgFFM for males and females respectively. P=0.38.

Fig 3.6: Femoral artery diameter (mm/kgFFM) in males (black) and females (white). Mean±SD values were 0.11± 0.02 and 0.12± 0.02 mm/kgFFM for males and females respectively. P= 0.24.

Fig 3.7: Arterial Constriction ((max-baseline)/range *100%). Mean±SD values were 46± 23%, 54± 15%, 75± 19%, and 66± 45% for endurance-trained supine, untrained supine, endurance-trained seated, and untrained seated conditions respectively. P< 0.05 for endurance-trained supine vs seated, p>0.05 for all other comparisons.

Fig 3.8: Arterial diameter (mean±SD) in posterior tibial artery. A: supine posture, endurance-trained subjects. B: seated posture, endurance-trained subjects. C: supine posture, untrained subjects. D: seated posture, untrained subjects. Each chart displays baseline, minimum, and peak
arterial diameter. P<0.05 for minimum diameter in A vs B and C vs D, p>0.05 for all other comparisons.
Figure 3.1
Figure 3.2
Figure 3.4
Figure 3.5
Figure 3.6
Figure 3.7
**Fig 3.8**

The figure shows four panels (A, B, C, D) each depicting a plot of Diameter (mm/kgFFM) against baseline, min, and max values. The plots illustrate variability across different conditions or time points, with error bars indicating the standard deviation or confidence intervals.
CHAPTER 4
SUMMARY AND CONCLUSION

Principal findings

THE MOST important outcome of the current study is the highly significant decrease in arterial range and increase in arterial constriction in an upright posture. Additionally, the absence of a strong effect of training on arterial range is balanced by the non-significant trend towards greater arterial constriction in the untrained group. If the experiment were repeated with a greater number of subjects, it is possible that a significant decrease in arterial constriction associated with chronic endurance training could be found.

Necessity of upright testing

The significant increase in arterial constriction observed in this study suggests that sympathetic tone is in fact increased in peripheral arteries under condition of increased hydrostatic pressure from gravity associated with upright posture. Further research is needed to establish the relationship between measurements of arterial control under more “functional” upright postures and overall arterial and cardiovascular health. Perhaps individuals with borderline cases of peripheral arterial disease that might appear normal under supine flow-mediated dilation testing would reveal a slight impairment during seated testing, thereby giving clinicians an earlier indication of a looming disease state.

Normalizing arterial diameter for fat-free body mass

There was a great deal of variability in arterial diameter in the present study, due mostly to variation in body size among subjects. Minimum diameters of the posterior tibial artery ranged
from 1.16 to 2.76 mm. While most researchers in this area examine more homogeneous samples and do not attempt to normalize arterial diameter measurements for differences in subject size, Krejza et al. (15) compared carotid artery diameter against a number of clinical measures such as height, weight, BMI, body surface area (derived from height and weight), etc and found that calculated body surface area was the best predictor of carotid artery diameter, although no measurements of body composition were employed.

In the present study, we elected to normalize arterial diameter by whole-body fat-free mass. This resulted in less variability between subjects for each measurement than normalizing by the fat-free mass of the limb in which the artery was measured. Normalizing arterial diameter by calculated body surface area could theoretically increase variability when comparing subjects with large differences in body composition.

Arterial range: an individual or group measure

Many measures in the current study, such as maximum diameter and arterial constriction, exhibited p values that were not significant, but displayed a trend towards statistical significance. In this respect, the current study may have been underpowered to detect real differences in arterial structure. Due to the variable nature of the populations under examination, both in terms of body size as well as sex and activity level, the measure of arterial range is a measure that has more utility in description of populations than as a clinical measure that can help determine the current state of an individual’s arterial regulation, despite a high degree of within-subject repeatability.

Limitations

There is an imbalance between males and females between groups. Given that sex may have resulted in a greater difference in arterial diameter than endurance training in this study, this
had a potential impact on our ability to detect small changes in arterial diameter and sympathetic control in response to a postural challenge between groups.

Although all subjects were asked to refrain from exercise on the day of testing, it is possible that there may have been lingering acute effects of vigorous exercise from the previous day. The possible acute effects and time course of exercise on measurement of arterial range have not been investigated.

This study was somewhat underpowered to detect some differences that did not quite reach statistical significance. If the total sample size was closer to 20 subjects per group, rather than ten, the conclusions may have been different. The biggest reason that the sample size was low in the current study was the stringent requirements for VO$_2$peak and training history. Approximately forty consent forms were signed for this experiment, but only twenty-one data points made it into the paper. Although several potential subjects dropped out before completion, or were excluded due to errors in the ultrasound test, the majority of these extra consent forms were excluded due to being too fit for the untrained group, but not fit enough for the endurance-trained group. If the criteria for inclusion in the untrained group were relaxed to include college-aged men with a moderate VO$_2$peak of perhaps 50-55 ml/kg/min, a much greater number of volunteers could have been utilized.

The state of ultrasound assessment of arterial range

This experiment was technically challenging. The use of a rack-and-pinion probe holder was essential to successful execution of the experiment, which required a minimum of one hour with the posterior tibial artery focused on the ultrasound display. In order to ensure a consistently-focused image of the artery that would allow for accurate diameter determination, both the subject and the researcher needed to remain very still throughout the experiment. If the
subject shifted at an inopportune time, or if the probe slipped, the outcome of the experiment could be ruined. In fact, the data from one subject had to be discarded due to an ill-timed sneeze coinciding with the post-hyperemic peak arterial diameter, rendering an accurate determination of arterial range impossible in that case. If examination of arterial range in a standing posture is attempted in the future, the greatest challenge by far will be unwanted movement of both the subject and the ultrasound probe.

**Future research**

Despite the current study being designed to find differences in arterial structure and function based on fitness level and exercise history, sex differences were revealed that were nearer to statistical significance than group differences based on fitness. The effect of sex on arterial range and function has not been examined, and has the potential to be significantly different. In addition, the large variability in body size and composition among the subjects in the current study may have had an inordinate effect on the variability of the data, despite attempts at normalizing arterial diameter for differences in lean body mass. Future researchers may consider selecting a sample that is more homogeneous in terms of body mass and composition.

In the current study, minimum arterial diameter in the seated posture was much greater than in the supine posture, despite the hypothesis that arterial tone is increased in upright postures. This may be due to the inability of venous blood to flow out of the leg after inflation of the cuff being exacerbated by gravity versus the supine posture. Techniques to allow for similar venous return between postures may need to be developed to ensure a more accurate comparison of arterial range between postures.
With the demonstration of small differences in arterial tone, the next step could be to measure arterial range in a supine versus standing posture, although the technical challenges would be greatly magnified over what is already a very technically challenging experiment.
CHAPTER 5

REFERENCES


