Aging is associated with an increase in arterial stiffness. Two different methods were used to evaluate arterial stiffness, pulse wave velocity and vasoactive range, in a young group and in an older group of males. The hypothesis was that a stiffer artery as measured by pulse wave velocity would have a narrower vasoactive range. Pulse wave velocity was measured over two segments. Vasoactive range was measured in the posterior tibial artery. The older group displayed significantly faster pulse wave velocities along all segments compared to the younger group. There was a non-significant trend for the older group to have a narrowed vasoactive range compared to the younger group, p = 0.096. There was no correlation between leg pulse wave velocity and posterior tibial vasoactive range suggesting range is not a measure of arterial stiffness. It can be concluded that age has a significant affect on arterial stiffness.

INDEX WORDS: Arterial stiffness, Pulse wave velocity, Vasoactive range, Vascular aging
PULSE WAVE VELOCITY AND VASOACTIVE RANGE: A COMPARISON BETWEEN HEALTHY YOUNG AND HEALTHY ELDERLY

by

MATTHEW STANTON REIFENBERGER

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PULSE WAVE VELOCITY AND VASOACTIVE RANGE: A COMPARISON BETWEEN HEALTHY YOUNG AND HEALTHY ELDERLY

by

MATTHEW STANTON REIFENBERGER

Major Professor: Kevin McCully
Committee: Elaine Cress
Randall Tackett

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2004
DEDICATION

In memory of Helen Madelyn Klocek Reifenberger (3/23/1916 – 5/29/04)

You will always be in our hearts and minds. Your kind hearted soul and life continue to inspire us all. May you rest in peace.
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CHAPTER ONE

Introduction

Cardiovascular disease has reached global epidemic levels. The impact of cardiovascular disease can be measured from a human standpoint and from a fiscal standpoint. According to the World Health Organization, cardiovascular disease accounts for 20% or 15 million deaths per year [1]. In 1999, 37.3% of all deaths in the United States were attributed to diseases of the heart and blood vessels [2]. By the year 2020, it is estimated that cardiovascular disease will surpass infectious disease as the world’s leading cause of death [3]. In financial terms, the American Heart Association estimated the cost of heart and blood vessel disease to be 368 billion for the year 2004 [4]. In short, cardiovascular disease has a very real and tragic impact on society.

Cardiovascular disease can take many forms including stroke, heart attack, coronary artery disease, congestive heart failure, hypertension and atherosclerosis. Risk factors for these diseases include inactivity, hypertension, excessive body fat, elevated triglycerides, diabetes, age, tobacco use, elevated LDL cholesterol, low HDL cholesterol, gender and family history [5]. With the exception of age, gender and family history most risk factors are preventable and reversible. It has been suggested that 90% of all coronary heart disease cases could be prevented if people practiced a healthy lifestyle [6].

One integral aspect of cardiovascular health is the vasculature. The vasculature is a dynamic system that can appropriately respond to the demands of the body. It also plays an important role in maintaining homeostasis throughout the body. Two important components of
vascular health are the endothelium and the degree of compliance or stiffness in the system. Many studies have looked at these variables in the brachial and carotid arteries but very few have looked at arteries in the lower leg such as the posterior tibial artery [7]. However, arteries in the lower leg might behave differently than those in the upper extremities. Especially, when one considers that most exercise interventions used to treat cardiovascular disease primarily involve the use of the legs during walking or cycling. Several studies have supported this by finding differences between upper and lower extremity arteries [8,9]. Furthermore, we feel we have a unique and worthwhile technique of quantifying vascular functioning through measures of vascular range and resting arterial constriction.

The purpose of this study is to measure vascular health in the lower leg via pulse wave velocity and ultrasound assessment of vasoactive range, resting arterial constriction and dilation. A key question is whether a stiffer artery has a decreased operating range. Also, the effect of aging on the vasculature was explored by recruiting and testing young (22.7 yrs) and older (65.8 yrs) subjects. Measures of vascular function, pulse wave velocity (PWV), arterial range, resting arterial constriction and dilation capacity were obtained and compared between young and old subjects.

**Study aims**

The aims of this study were to:

1. Quantify global arterial stiffness using pulse wave velocity in young and old subjects.
2. Quantify vessel health, in terms of vasoactive range, resting arterial constriction and flow mediated dilation.
3. Correlate pulse wave velocity to range.

4. Relate lower limb volume to baseline arterial diameter.

**Hypotheses:**

It is hypothesized that:

1. Older subjects will display faster pulse wave velocities, suggesting stiffer arteries, and a smaller vessel operating range when compared to young subjects.

2. Younger subjects will have a greater ability to dilate in response to increased flow compared to older subjects.

3. A stiffer artery, as measured by pulse wave velocity will have a diminished operating range.

4. There will be a relationship between lower limb volume and baseline diameter such that individuals with larger lower limb volumes will have larger resting diameters.

**Significance of the study**

The significance of this study is two-fold. First, two different methods of measuring arterial functioning will be investigated. One method, pulse wave velocity, has been widely used in the past to provide a global index of arterial stiffness. The second method, unique to us, uses ultrasound to directly measure and characterize properties of vessel functioning such as operating range, resting arterial constriction and dilation capacity. Stiff, unhealthy arteries that are expected in an older population might display diminished operating range and dilating capacities. Using ultrasound to characterize vessel properties will provide direct site specific information useful to understanding arterial functioning.
The second significant aspect of this study is to compare the measures of vascular functioning between young and old subjects. It is widely accepted that aging increases the stiffness of arteries. However, the operating range of vessels has not yet, to the author’s knowledge, been documented. This study will document range in the posterior tibial artery of young and old subjects. Finally, arteries in the leg, such as the posterior tibial, have been overlooked and emerging evidence suggests its value.
CHAPTER TWO

Review of Related Literature

Gender and cardiovascular disease

Males have a lower life expectancy than females in part because of increased incidence of cardiovascular death in middle age men [10]. Gender remains a cardiovascular risk factor even after removing influences such as cigarette smoking and hypertension. By the age of 60, one in every five men and one in 17 women have some form of coronary heart disease [11]. Endothelial health starts to decline in males before females presumably due to the protective effect of estrogen on the female vasculature [12].

Arterial health

The focus of cardiovascular disease has become the arterial wall and in treating many cardiovascular maladies the arterial wall is the target [13]. Arterial health consists of arterial stiffness determined by the elastin/collagen ration as well as the endothelium’s ability to maintain vascular homoeostasis through its release of substances affecting vascular smooth muscle. The rest of this review will focus on these two topics and how each changes with age and exercise.
Arterial stiffness

The arterial system is designed to provide a continuous supply of oxygen rich blood to peripheral tissues by retaining a portion of each stroke volume for release during diastole [14]. This is accomplished by the elastic recoil of arteries during diastole. This process is also responsible for preventing abnormal high pressure situations from occurring in the arteries. A very compliant artery will be able to easily expand during systole keeping pressure low. When vessels lose compliance (stiffen), blood flow becomes intermittent and the arteries are subjected to higher pressures. Age related stiffening associated with the aorta occurs after the age of 60. This stiffening process leads to less diastolic recoil in the aorta which in turn leads to a decreased aortic contribution to forward flow [15]. Consequences of a less compliant artery also include of left ventricular hypertrophy, stroke and atherosclerosis. Arterial stiffness is a non-descript term that is generally used to signify the inverse of compliance; compliance being the change in the internal diameter of an artery that accompanies a given change in intravascular pressure. Stiffening of the arteries is now thought to be the root cause of a number of cardiovascular diseases [16]. The stiffness of an artery has been proposed to depend on structural changes as well as ionic cellular changes. Structurally, the most important changes include a fraying and fracturing of elastin fibers, decrease in the density of arterial wall elastin, increase collagen content and an increase in collagen molecule cross-linking [17]. Ionic cellular changes include of a decrease in magnesium levels and increases in cytosolic free calcium levels [18]. The degree of vascular smooth muscle constriction also influences compliance.
**Arterial stiffness and aging**

Aging has been shown to be the primary determinant of central artery stiffness [19]. Smulyan *et al.* have shown an increased aortic pulse wave velocity from 1000 cm/sec to 1300 cm/sec in a cohort of males less than 40 years of age and greater than 55 years of age respectively [20]. Cross-sectional studies have shown that aortic pulse wave velocity increases about 10 cm/sec per year [19]. Similarly, Tanka *et al.* have observed a 40% decrease in carotid artery compliance between the ages of 25 and 75 using a technique that involves the combination of ultrasound and tonometrically obtained arterial pressures to measure compliance [21]. The structural changes concerning elastin and collagen, previously mentioned, are responsible for this decrease in compliance with age. Arteries also enlarge and dilate with age [22].

**Arterial Stiffness and exercise**

Epidemiological studies have found that people who exercise on a regular basis are at decreased risk for developing cardiovascular disease [23, 24]. Exercise positively affects blood pressure, glucose regulation, body composition, plasma lipids, and endothelial function [25]. Recently, it has been shown that regular exercise may attenuate the loss of arterial compliance that accompanies age [17, 21]. Tanaka *et al.* found that central arterial compliance was approximately 40% higher in older endurance trained subjects compared to older sedentary counterparts [21]. The exact mechanism for this attenuation of age related compliance in endurance trained subjects is unknown at this point in time. However, several mechanisms have been proposed. The widened pulse pressure that occurs during exercise might sufficiently stretch collagen fibers which could remodel the vessels to increase compliance [26]. Additionally, Matsuda *et al.* found that exercised rates contained increased amounts of aortic elastin with less
calcium deposits present [27]. Regular exercise may increase arterial compliance by enhancing the sympathoinhibitory effect of nitric oxide thus overriding the chronic suppressive influence of the sympathetic system [21]. Increased sympathetic activity has been shown to decrease compliance by affecting vascular smooth muscle in the arterial wall [28].

Measurement of arterial stiffness

A number of methods have been developed to quantify arterial stiffness [29-31]. Perhaps the oldest and most widely used technique of evaluating arterial stiffness is pulse wave velocity. As the name implies, pulse wave velocity is a measure of the velocity of the pulse as it travels down the arterial tree. Measurement of pulse wave velocity provides a global indication of arterial stiffness in a particular vascular bed. Pulse wave velocity is related to the Young’s Elastic Modulus of a thin walled homogenous elastic tube by the Moens Korteweg equation (pulse wave velocity = \sqrt{\frac{E \times h}{2r \rho}}) where E is the elastic modulus, h/2r is the wall thickness divided by the diameter and \rho is the viscosity of blood [32].

When using pulse wave velocity to measure arterial stiffness, the Arterial Stiffness Task Force III recommends the following procedures [33]:

1. Subjects rest for a minimum of 10 minutes prior to testing.
2. Subjects abstain from caffeine and alcohol 3 hours and 10 hours prior to testing.
3. Subjects remain in resting conditions neither talking nor sleeping during the testing.
4. Measurements made in a quiet room at room temperature
**Endothelial health**

The endothelium consists of a smooth, single layer of endothelial cells which line the inner surface of the vasculature. Endothelial cells function in an autocrine, paracrine and endocrine fashion, regulating a number of processes that include vasoreactivity, smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, immune response and thrombolysis [34]. Endothelial function has been shown to predict subsequent cardiovascular risk as well as be impaired by age, gender, hypertension, smoking, diabetes and other known coronary risk factors [34, 35]. The endothelium has been recognized for the important role it plays in regulating vascular reactivity through the release of vasodilators (nitric oxide, prostacyclin, bradykinin and hyperpolarizing factor) and vasoconstrictors (endothelin, thromboxane) [36]. Of these, nitric oxide has been widely studied. Formerly known as endothelial derived relaxing factor, nitric oxide was isolated in 1998 by Robert Furchgott. Nitric oxide plays an important role in the autoregulation of blood flow. Nitric oxide, a gas, is synthesized in the endothelium by the conversion of L-arginine to nitric oxide by nitric oxide synthase. Nitric oxide is released from the endothelium, in response to elevations in vascular shear stress, whereby it diffuses into the smooth muscle causing smooth muscle relaxation and vasodilation. Specifically, nitric oxide increases smooth muscle cell cyclic guanosine monophosphate [37]

Due to the important nature of the endothelium in maintaining cardiovascular homeostasis, researchers have developed a non-invasive test of endothelial function termed flow mediated dilation (FMD). Flow mediated dilation tests the ability of the endothelium to appropriately respond to increases in shear stress created by increased flow. Increased flow, in this situation, is achieved by using cuff occlusion and cuff release to create a reactive hyperemic
situation. An artery with a healthy endothelium will dilate in response to increased blood flow. Flow mediated dilation is expressed as a percentage increase from baseline diameter. A 10.4 % flow mediated dilation value has been suggested as the cut off point for distinguishing endothelial dysfunction from a healthy endothelium, although the magnitude of FMD varies with the method used to measure it [38]. This same study found that a population of 253 normotensive healthy subjects with no cardiovascular risk factors had a mean flow mediated dilation of 14% while subjects with at least one cardiovascular risk factor had a mean flow mediated dilation of 7% [39].

Numerous studies have investigated endothelial dysfunction and flow mediated dilation. Anderson et al. have shown that patients with coronary artery endothelium dysfunction had a significantly impaired brachial flow-mediated vasodilator response compared to controls (4.8±5.5% vs 10.8±7.6%) [40].

**Endothelium and age**

Aging has been associated with impaired endothelium/nitric oxide dependent vasodilation in men at a rate of .21% per year after the age of 40 [12]. These reductions have been observed both in the brachial and coronary arteries. Increasing age was associated with a progressive decrease in vasodilation in response to acetylcholine [40]. One proposed mechanism for this age related decrease in vasodilation is a reduced bio-availability of nitric oxide perhaps as a result of alteration in the L-arginine – nitric oxide pathway via excess oxygen derived free radicals [41, 42]. The age-related loss of endothelial functioning will contribute to the increased risk of atherosclerosis and coronary heart disease. It can be concluded that endothelium-dependent vasodilation declines steadily with increasing age in healthy human subjects.
Endothelium and exercise

Physical exercise via increased blood flow and shear stress, results in increased nitric oxide bioavailability as well as increased nitric oxide synthase activity [43]. Cell culture studies have shown that nitric oxide synthase mRNA is up-regulated in endothelial cells exposed to increased flow [44]. Human studies, such as by Clarkson et al, have shown that 10 weeks of regular physical activity in young men improved brachial flow mediated dilation from 2.2% (pre-training) to 3.9% (post-training), possibly contributing to the beneficial cardiovascular effects of exercise [45]. Cross-sectional data shows that trained older men display a greater vasodilatory response compared to their sedentary counterparts when intra-arterially exposed to acetylcholine demonstrating that endothelium dependent vasodilatation was well preserved with age in men who regularly performed endurance exercise [46]. The time course for improved flow mediated dilation has also been studied. Allen et al. observed a significant increase in brachial flow mediated dilation after only 4 days of training [47].

Vasoactive range

Vasoactive range is a term used to describe the operating range of a vessel. The operating range of a vessel depends on the interplay between the endothelium, smooth muscle and the elastin/collagen content of the artery wall. Vasoactive range is measured using high resolution ultrasound to image changes in artery diameter during both ischemic and hyperemic conditions as similarly done during tests of flow mediate dilation. The main difference being the length of cuff occlusion is ten minutes as opposed to five minutes. Theoretically, with a ten minute cuff occlusion, the artery is not only reacting to flow but also to metabolites that have built up during
the prolonged ischemic/hypoxic period. A minimum diameter is obtained during the ischemic condition while a maximum is obtained during the first minute or two of cuff release. Measurements of the ischemic artery are considered to reflect intrinsic tone while measurements during hyperemia reflect maximal vasodilatory capabilities. Conceivably, an artery with a decreased operating range might potentially represent a stiffer artery. In one of the few studies investigating vasoactive range Harris et al. found that subjects with peripheral vascular disease had a decreased brachial artery operating range [48]. The same study also found that old normals and subjects with peripheral vascular disease had a decreased ability to vasoconstrict from baseline compared to young normals (46.5% young normals, 29.2% old normals and 15.3% peripheral arterial disease).

**Physics of ultrasound**

Ultrasound has become a popular medical/scientific instrument since its advent in the early 1950s [49]. The idea of ultrasonic imagining is relatively simple; emit sound waves into the body and collect the reflected sound waves whereby producing an image of the body. The sound wave will travel through the different tissue types and structures of the body occasionally reflecting back towards the ultrasound transducer. Based on these echoes an image of the body is produced.

Ultrasound refers to sound waves that have a frequency of greater than 20,000 Hz and are outside our range of hearing as humans. Ultrasound and “sound” in general describes pressure vibrations that travel through a medium such as air, body tissue or water. These vibrations are formed by transducers. To generate ultrasound waves, those greater than 20,000 Hz, the transducer needs to vibrate at a high frequency. Piezoelectrics are generally used to produce the
ultrasonic waves. Useful Piezoelectrics generators of ultrasound are made of ceramic discs that vibrate at high frequencies by applying a time varying voltage across opposing sides of the ceramic.

The frequencies used in ultrasound imaging are generally from 2,000,000 Hz – 15,000,000 Hz. In ultrasound, the wavelength of the sound waves determines the spatial resolution. Wavelength is related to frequency by the wave equation,

$$\lambda = \frac{V_s}{f}$$

where \(\lambda\) is wavelength (m), \(V_s\) is speed of sound in soft tissue \(\approx 1570\) m/sec, and \(f\) is frequency (Hz) [50]. These frequencies correspond to wavelengths of 0.785 mm and 0.105 mm respectively. These frequencies are typically used because they have associated wavelengths small enough to reflect off structures less than 1 mm. Apart from the spatial resolution of ultrasonic waves, it is also important know the penetration depth of the wave into a material. A practical depth for this diagnostic depth in soft tissue is roughly 200 times the wavelength of the incident sound wave.

Images are formed by detecting echoes reflected from an interface of two tissue types that have different acoustic impedance values. Acoustic impedance refers to what fraction of a sound wave is reflected at the interface and is a product of the medium’s density and the speed of sound in the medium. The greater the difference in acoustic impedance between the two tissue types, the greater the intensity of the reflected sound wave. The echo intensity received back from each interface can be electronically reconstructed and displayed. The echo intensity can then be mapped into a pixel brightness and viewed in a monitor. Substances with large differences in acoustic impedance will produce large echo intensities and subsequently will appear very bright on the screen. Conversely, substances with small differences in acoustic impedance will produce
small echo intensities and will appear lighter grey. This conversion between echo intensity and pixel intensity is referred to as a grey scale image.

**Baecke Questionnaire of Habitual Physical Activity**

Measurement of physical activity is an important aspect of research concerning health in our field. Questionnaires are often the least bad method when subject sizes are large and/or it is impractical to directly measure energy expenditure or VO₂max. The Baecke Questionnaire of Habitual Physical activity is one such questionnaire. In this questionnaire, subjects respond to 16 questions concerning their activity levels in the following three arena: work, sport and leisure [51]. Based on their responses, a score is calculated for each arena. A cumulative score is then calculated by adding each of the three component scores. A higher score indicates a more physically active subject. This questionnaire has been found to be reproducible between a first test and a 1 month retest [52]. This same study found a significant correlation between the Baecke total index score and VO₂max. In another study, Pols et al found a significant relationship between the Baecke questionnaire and energy expenditure over three days as measured by an activity diary [53].
CHAPTER THREE

Pulse Wave Velocity and Vasoactive Range: a comparison between healthy young and healthy elderly

Introduction

Cardiovascular disease claimed 39.4% of all deaths in the year 2000 and continues to be the number one killer in the United States for both men and women and while cardiovascular disease mortality trends have reversed since the mid 80’s, males continue have a higher cardiovascular disease death rate [1]. Both endothelial function and arterial stiffness play important roles in maintaining cardiovascular homeostasis and both have been implicated in cardiovascular disease progression [2, 3]. Up to now, few if any, studies have investigated both endothelial function/dysfunction and arterial stiffness. A logical link exists when one considers that factors that impair endothelial health are also thought to increase large artery stiffness and that the large muscular component of arteries are, in part, under control of the endothelium.

Aging has been shown to have a significant impact on the stiffness of arteries [4-6]. Aging in and of it self, brings about structural changes to the vasculature. The most important changes consist of a fraying and fracturing of elastin fibers, decreases in the density of arterial wall elastin, increases in collagen content and the manner in which these collagen molecules cross-link [7]. Endothelial health starts to decline in males before females presumably due to the protective effect of estrogen on the female vasculature [8]. Healthy older males were recruited and compared to healthy young males in order to study the effects of aging on the vasculature.
This study attempts to investigate vascular function via a traditional method, pulse wave velocity, and an emerging technique vasoactive range. Pulse wave velocity, a widely used technique to measure arterial stiffness has been shown to increase with aging [9, 10]. Vasoactive range refers to the operating range of the vessel elicited by subjecting the artery to a period of ischemia, to achieve a minimum diameter, and hyperemia to achieve a maximum diameter. A lower vasoactive range may suggest a stiffer artery unable to relax. A study (unpublished) from our lab has shown that vasoactive range is attenuated in spinal cord (0.36 ± 0.28 mm) injured individuals and improves with 18 weeks of neuromuscular electrical stimulation induced resistance training (0.94 ± 0.40 mm). A stiffer artery, as measured with pulse wave velocity, was expected to have a narrower vasoactive range. A standard indicator of endothelial health, flow mediated dilation, was also measured.

Methods

Subjects

A total of 35 males were recruited and placed into either a young group or an old group based on age (N=20 in the young group, N=15 in the old group). Table 1 contains group physical characteristics. All subjects reported the absence of any smoking history, cardiovascular disease, diabetes, polycythemia, anemia or lower leg claudication pain. The study was conducted with the approval of the Institutional Review Board at the University of Georgia and all subjects provided written informed consent.
Tests

All testing was done between the hours of 7 a.m. and 11:30 a.m. to minimize any circadian affects. Each subject reported once to the lab for testing and responded negatively to questions concerning consumption of caffeine, alcohol or high fatty foods 10 hours prior to testing. Subjects responded to the Baecke Questionnaire of Habitual Physical Activity to assess activity levels. Height and weight was measured and body mass index calculated. Subjects then rested quietly on the examination table for a minimum of 10 minutes. Systolic pressure, diastolic pressure, mean arterial pressure and heart rate were measured and recorded using an automated blood pressure machine (Datascope, Mahwah, NJ).

Pulse wave velocity measurement

Pulse wave velocity was measured using ECG leads, a blood pressure cuff wrapped around the right ankle and another blood pressure cuff placed around the thigh, see figure 1. All three outputted into Biopac hardware in conjunction with Acknowledge Software set to collect at 300 samples per second. Specifically, the ankle cuff was placed proximal to the right medial malleolus. The thigh cuff was placed as proximal on the upper leg as possible. Pressure waves were attained sequentially by inflating each cuff to 60 mmHg for one minute. A tape measure was used to measure the following distances: suprasternal notch to middle of thigh cuff ($d_1$) and middle of thigh cuff to middle of ankle cuff ($d_2$). This setup allowed two different pulse wave velocity values to be calculated: aortic pulse wave velocity and leg pulse wave velocity. Aortic pulse wave velocity (cm/sec) was calculated by dividing

1. the aforementioned distances ($d_1$) measured in centimeters by
2. the average time difference in seconds between the foot of the pressure wave and the peak of the QRS (t₂) wave over six cardiac cycles. 0.05 seconds was subtracted to take into account isovolumetric contractions time.

Leg pulse wave velocity values were calculated by dividing

1. the distance from the middle of the thigh cuff to the middle of the ankle cuff (d₂) by
2. (t₃) the difference in time values (t₁ – t₂)
**Figure 1:** Diagram of pulse wave velocity measurement. Distances ($d_1$, $d_2$) were measured in centimeters with a tape measure. Time 2 ($t_2$), was found by subtracting time point A from time point B and was used in conjunction with $d_1$ in calculating aortic pulse wave velocity. Time 3 ($t_3$), found by subtracting $t_1$ from $t_2$, in conjunction with $d_2$, was used to calculate leg pulse wave velocity. Time values used were an average of those occurring over six cardiac cycles.

**Baseline, minimum and maximum arterial diameter measures**

With the subject resting in the supine position, the posterior tibial artery was imaged 3 – 5 cm proximal to medial malleolus using an ultrasound unit with a 9-13 MHz linear-array ultrasound transducer set in B mode (General Electric LogiQ 400CL, Rainbow City, Al). Magnification and focal zone settings were adjusted to optimize picture quality. All pictures were taken during the end of diastole which was determined visually. Pictures taken during the test were analyzed using custom-made, semi-automated wall detection software (Labview 6i, Austin, TX).

To measure baseline, minimum and maximum diameter the following sequential protocol was used: five minutes of resting conditions to establish baseline diameter, ten minutes of cuff ischemia to determine a minimum diameter and five minutes of hyperemia to determine maximum diameter. Figure 2 is a representative example of images captured during each period. To create the ischemic/hyperemic conditions a pneumatic tourniquet (D.T. Hokanson Inc.) placed around the right thigh, proximal to the knee as well as the measurement site, was rapidly inflated to 100 mmHg above systolic blood pressure. Use of a proximal cuff to induce hyperemia has been shown to have greater effect sizes [11]. Pictures were taken often enough to sufficiently
capture the responsiveness of the artery as seen in figure 3. From this graph, baseline diameter, minimum diameter, maximum diameter and vasoactive range were determined.

Following five minutes of hyperemia resulting from release of the ten minute cuff, a standard flow mediated dilation test was performed. This test was performed similarly with exception that a five minute cuff inflation period was used instead of the ten minutes previously used to determine range.

**Calculations**

Baseline diameter was accepted to be the average of all diameters taken during the five minute resting baseline period. Minimum and maximum diameters were found by averaging the three smallest consecutive diameters and the three largest consecutive diameters respectively. The following three equations were used to calculate range, resting arterial constriction and flow mediated dilation.

\[
\text{vasoactive range} = \text{maximum diameter} - \text{minimum diameter}
\]

\[
\text{resting arterial constriction} = \left(\frac{\text{max} - \text{baseline}}{\text{range}}\right) \times 100
\]

\[
\text{flow mediate dilation} = \left(\frac{\text{max} - \text{base}}{\text{base}}\right) \times 100
\]

Vasoactive range defines the operating range of the vessel. Resting arterial constriction describes the degree of constriction an artery exhibits at rest and is expressed as a percentage of range. Flow mediated dilation refers to the dilation following the five minute cuff and is expressed as a percentage of baseline.
Lower limb volume was measured using a lower limb volumeter. Briefly, subjects submerged their leg up to the head of the fibula in a partial leg volumeter (Volumeter Unlimited, Inc.) filled with water. The displaced water was collected, weighed and from this volume was calculated.

**Statistical analysis**

All values are reported as means ± standard deviation. Independent samples t-tests (SPSS) were conducted to compare means among variables between the young group and the older group. Analysis were conducted with statistical significance accepted at $\alpha = .05$. Leven’s test of homogeneity was conducted to test for homogeneity of variance. When appropriate correlations were run with strength of relationship reported via Pearson’s Correlation Coefficient.
Figure 2: Picture A (top) taken during baseline (diameter = 2.55 cm), picture B (middle) taken during ischemia represents a minimum diameter (diameter = 2.20 cm), Picture C (bottom) taken during hyperemia represents a maximum diameter (diameter = 3.06 cm). In all cases bright white parallel lines represent arterial wall with lumen in between
**Figure 3**: Representative example of the time course of diameter change during five minutes of baseline, ten minutes of cuff ischemia and 5 minutes of cuff release. Time point zero indicates the release of the cuff. Minimum and maximum diameters were found by averaging the three smallest consecutive diameters or the three largest consecutive diameters respectively. Range was then calculated as the difference in these measures. Although not represented above there was another cuff and release period following hyperemia to calculate flow mediated dilation.

**Results**

The physical characteristics of all subjects are presented in table 1. There were no group differences between height, weight, BMI, mean arterial pressure resting heart rate and Baecke Questionnaire of Habitual Physical Activity score. There was a significant difference in age $P < 0.001$ between groups. Baecke Score represents the average score on a self reported measure of physical activity (Baecke Questionnaire of Habitual Physical Activity). A higher score represents a higher level of habitual physical activity with the highest score possible being 15. The range of
Baecke scores in the young group was 6.1 to 10.3. The range of Baecke scores in the older group was 4.75 to 11.5. Mean arterial blood pressure ranged from 74 to 104 mmHg in the young group and 83 to 117 mmHg in the older group. Average systolic and diastolic blood pressure in the young group was $117 \pm 8$ and $67 \pm 8$ mmHg respectively. In the older group average systolic and diastolic blood pressure was $129 \pm 11$ and $77 \pm 7$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)*</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI</th>
<th>MAP (mmHg)</th>
<th>Resting HR (bpm)</th>
<th>Baecke Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>22±2.6</td>
<td>1.78±.01</td>
<td>79.8±19.4</td>
<td>25.12±5.4</td>
<td>87.2±7.6</td>
<td>61.2±10.1</td>
<td>8.3±1.5</td>
</tr>
<tr>
<td>Old</td>
<td>68±8.6</td>
<td>1.75±.01</td>
<td>80.8±15.8</td>
<td>26.25±3.9</td>
<td>99.0±9.6</td>
<td>66.5±7.8</td>
<td>7.1±2.1</td>
</tr>
</tbody>
</table>

**Table 1:** Physical characteristics for N=20 young subjects and N=15 older subjects. Values are mean ± standard deviation. * P<0.001

An independent two-tailed T-test was conducted to evaluate differences in baseline diameters between groups. The results, seen in figure 4, show that mean baseline diameters were $2.65 \pm 0.52$ mm in the young group and $2.86 \pm 0.71$ mm in the older group. The corresponding p value was 0.323 indicating no difference between groups. However, there was a significant correlation between resting diameter and age ($r = 0.6$, $p = 0.023$) among the subjects from the older group, as seen in figure 5. Furthermore, there was no correlation between resting arterial diameter size and lower limb volume, $r = 0.12$, $p = 0.476$ (not shown) among all 35 subjects or resting arterial diameter size and activity level, $r = 0.26$, $p = 0.252$ (not shown) among the younger subjects. Similarly, although not shown, there was no relation between resting arterial diameter size and activity levels amongst the older group $r = -0.301$, $p = 0.295$. 

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**Figure 4:** Average baseline resting diameter in young, $2.65 \pm 0.52$ mm, and old, $2.86 \pm 0.71$ mm groups. $P = 0.323$ indicating no difference between groups.

**Figure 5:** Correlation between baseline diameter (cm) and age (yrs). Data point include only older group.
An independent two-tailed T-test was conducted to evaluate whether pulse wave velocity was faster, indicating stiffer arteries, in the legs of the older group. The results, shown in figure 4, indicate that the mean pulse wave velocity value in the older group (M=1292 ± 155 cm/sec) is greater than the mean pulse wave velocity value in the young group (M=1164 ± 198 cm/sec). The respective p value for this comparison is p = 0.05. Cohen’s D effect size is 0.72 and the 95% confidence interval on the mean difference is -0.54 to 258 cm/sec. Post hoc power was calculated to be 0.66. Although not graphically shown the young group had an average aortic pulse wave velocity of 677 ± 93 cm/sec and the older group 1415 ± 198 cm/sec. The difference in PWV between age groups remained significant when analysis of covariance (ANCOVA) was used to remove the affect of mean arterial blood pressure (p < 0.001). The resulting p – value was < 0.001. The effect size was 3.2 and Post hoc power was calculated to be 1.0

**Figure 6**: Average pulse wave velocity values in leg. (**) P = 0.05. The older group had faster values indicating stiffer arteries.
Correlations between age and leg pulse wave velocity/aortic pulse wave velocity among the older group are seen in figures 7 and 8. The Pearson Correlation Coefficient for leg pulse wave velocity and age was \( r = -0.073 \) with a \( p \) value of 0.803. The Pearson Correlation Coefficient for aortic pulse wave velocity and age was \( r = 0.63 \) with a \( p \) value of 0.022. Activity levels, as measured by the Baecke Questionnaire of Habitual Physical Activity, were not significantly correlated to any of the pulse wave velocity measurements.

**Figure 7:** Correlation between leg pulse wave velocity and age, \( r = -0.073. \ p = 0.803. \)
Figure 8: Correlation between aortic pulse wave velocity and age, $r = 0.63$, $p = 0.022$

While not graphically represented, results of posterior tibial range, resting arterial constriction and flow mediated dilation are shown in table 2. There was a trend for the older group to have a narrower vasoactive range. Average range in the young group was $0.97 \pm 0.38$ mm while $0.78 \pm 0.19$ mm in the older group. This resulted in a $p$ value of 0.096 and an effect size of 0.63. Post hoc power was calculated and was equal to 0.56. There was a more substantial difference in resting arterial constriction between groups, $43 \pm 13.85\%$ in the young group and $30 \pm 15.52\%$ in the older group. The resulting $p$ value for this relationship was 0.017 with an effect size of 0.82. Average flow mediated dilation values were $12.1 \pm 4.9\%$ in the young group and $9.2 \pm 6.1\%$ in the older group resulting in a corresponding $p$ value of 0.155. Post hoc power was calculated and was equal to 0.44.
<table>
<thead>
<tr>
<th>Range (mm)</th>
<th>Resting Arterial Constriction (%)</th>
<th>FMD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>old</td>
</tr>
<tr>
<td>mean</td>
<td>0.97</td>
<td>0.78</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.38</td>
<td>0.19</td>
</tr>
<tr>
<td>p-value</td>
<td>p = 0.096</td>
<td>p = 0.017</td>
</tr>
<tr>
<td>effect size</td>
<td>0.63</td>
<td>0.82</td>
</tr>
<tr>
<td>95% C.I mean difference</td>
<td>-0.03 to +4.1</td>
<td>+2.48 to +23.14</td>
</tr>
</tbody>
</table>

Table 2: Mean, standard deviation (Std Dev), p value, effect size and the 95% confidence interval mean difference for range, resting arterial constriction and flow mediated dilation (FMD).

The correlation between vasoactive range and leg pulse wave velocity is shown in figure 9. The Pearson Correlation Coefficient was $r = -0.44$ with a p value of 0.136.

![Figure 9: Relationship between vasoactive range (cm) and Leg pulse wave velocity (cm/sec).](image)

Data points include all subjects.
Discussion

The purpose of this paper was to compare two methods of evaluating peripheral arteries, pulse wave velocity and measurements of arterial range and constriction. Young and older subjects were chosen based on previously demonstrated age-related changes in vascular stiffness, autonomic vascular control, and vascular reactivity [12-14]. Similar to previous studies, we found an effect of age on arterial diameter [15]. While young and older subjects were not statistically different in arterial diameter, there was a significant increase in arterial diameter with increasing age in the older group. We did not see any relationship between arterial diameter and either limb volume or habitual activity level. We had hypothesized that larger men would have larger arteries. However, the crude measurement technique of lower limb volumetry presently used which does not distinguish between fat and muscle. Future studies should incorporate more advanced measurement techniques (MRI or DEXA) which are able to distinguish between fat and muscle. We also hypothesized that arterial size would be related to physical activity levels. The lack of a relationship with physical activity level could reflect relatively small differences in activity level between our subjects, or that the posterior tibial artery serves the foot and is not that sensitive to activity levels. In previous studies we have found that spinal cord injured patients do not have reduced posterior tibial arteries compared to able bodied subjects, despite have 40% smaller femoral arteries (unpublished).

Average leg pulse wave velocity tended to be slightly faster in leg arteries of older subjects compared to their younger counterparts. Faster pulse wave velocity values indicate stiffer arteries, a result consistent with the literature. Most of the previous studies have used inactive subjects, and our more active subjects maybe expected to show smaller differences between age groups due to the positive effect of exercise on PWV measurements. Interestingly,
we found larger age related differences in our measure of central arterial PWV than in our peripheral measurement. Our correlation of aortic pulse wave velocity and age is in agreement with Avio et al. who found a similar correlation coefficient of 0.67 between aortic pulse wave velocity and age in urban Chinese [13]. Differences in arterial stiffness between central arteries and peripheral arteries are most likely related to differences in the elastin-collagen smooth muscle proportions found along the arterial tree as proposed by Van der Heijden-Spek et al. [16].

There was a non-significant trend for vasoactive range to be smaller in the older subjects. It should be noted that there was a fairly substantial effect size that was offset by the high degree of variability. Moreover, this small difference in posterior tibial vasoactive range bares a resemblance to the small difference seen in leg pulse wave velocity and again most likely reflects the small degree of age associated arterial stiffening in the peripheral arteries. It can not be overlooked that these subjects were all healthy and fairly active. Recent results from our lab (unpublished) found that spinal cord injured subjects had a posterior tibial artery range of 0.36 ± 0.28 mm and that their range improved to 0.94 ± 0.40 mm following 18 weeks of neuromuscular electrical stimulation induced resistance training. This result coupled with ours might indicate that posterior tibial artery range is more affected by disease and inactivity than by age. A study by Harris et al. found a similar non significant reduction in the range of the brachial artery in healthy old normals compared to young normals but a large difference when comparisons were made to a group diagnosed with peripheral arterial disease [17]. This also suggests that range might be more drastically changed with disease.

It was hypothesized that a negative correlation exists between pulse wave velocity and vasoactive range. That is, subjects' with a faster pulse wave velocity, indicating a stiffer artery, would have a narrower vessel operating range. There appears to be no such relation. This finding
suggests that vasoactive range is not a measure of arterial stiffness as we had originally thought. Despite this finding, vasoactive range is still an important variable to measure especially in understanding and interpreting vascular remodeling situations and changes to resting arterial diameter.

There was no relation between indices of vascular health, either pulse wave velocity measures or vasoactive range, and the total score or sports index score from the Baecke Questionnaire of Habitual Physical Activity (not shown). This is in contrast to Tanaka et al, who demonstrated that central arterial compliance in endurance trained middle and older aged men was higher when compared to their age matched sedentary and recreational active counterparts [18]. There are several possible explanations for this. One is the inevitable inaccuracy associated with measuring habitual physical activity especially when the technique is a questionnaire. Second is that the greatest benefit in terms of preventing the age related loss of arterial compliance is seen in highly trained endurance individuals. These are individuals that vigorously trained 5 times per week. "Recreationally" active individuals seem to miss out on this benefit. This study focused on the recreationally active older male. Another possibility was that we chose to measure peripheral arterial stiffness while Tanaka et al measured central arterial stiffness. It maybe that central arterial stiffness reflects a different aspect of arterial circulation, that of central regulation of blood pressure, while peripheral arterial stiffness may reflect peripheral arterial health. As our older subjects were all physically active, particularly with their legs, it may be that their peripheral arterial stiffness should be similar to younger subjects.

The older group displayed a decreased flow mediated dilation response. This result is in agreement with Celermajer et al. who found that aging is associated with endothelial dysfunction leading to a attenuated flow mediated dilation response [19]. The 9.2% flow mediated dilation,
seen in the older group, is generally considered to be in the healthy range. It is slightly below the 10.4% cut point suggested by Accini et al. in discriminating between subjects with and without cardiovascular risk factors [20]. However, we feel our value is near enough and represents a healthy endothelium.

**Limitations**

As with all pulse wave velocity measurements path length distances used in calculations might not accurately represent the actual path length of the vessel. The measured path lengths for each segment agreed between groups within a couple of centimeters (not reported). It is assumed that the error involved in this is equal between groups. Another limitation is that isovolumetric contraction time was assumed to be 0.05 seconds for all subjects which might confound the aortic pulse wave velocity values, although our values agree with the literature. Also, the relatively small sample sizes can make conclusions about the population and relationships tenuous.

**Conclusions**

Resting posterior tibial artery diameter was related to age but not lower limb volume or habitual physical activity levels. Pulse wave velocity increased with age indicating an age related increase in arterial stiffness for central arteries and those in the leg. However, the effect size was much greater in the central arteries. Leg pulse wave velocity did not correlate with posterior tibial vasoactive range suggesting range might not be an indicator of arterial stiffness. Flow mediated dilation was less in the older group but still near a “healthy” range. The older group represented was recreationally active and small differences in vascular health were expected.
Futures studies should investigate said relationships in diseased populations were vascular health differences might be more pronounced.

References


CHAPTER FOUR
Concluding Remarks

It is clear that vascular health will continue to be an important area of research. However, it is not clear which indices of vascular health will become standard. The complexities of the vascular system are such that no one method accurately captures and reflects the working state of vessels, e.g. endothelial status, arterial compliance, blood flow, vascular inflammation markers. Furthermore, these variables are changing throughout the length of the arterial segment compounding the issue of a test for vascular health. At the present state if one is interested in accurately characterizing arterial function a whole battery of tests is needed.

Overall I believe the study went well. However, there are a few areas I would like to address. Recruitment of subjects was more difficult than expected and ultimately I was unable to obtain the subject pool I had originally proposed. I take away from this experience that in order to effectively recruit the population in interest, strong ties with that community of individuals is needed. It seems these bonds can be made first hand, between the primary study investigator and the community, or second hand via another individual, doctor, social worker etc., who professionally has contact with the community of interest. I believe I lacked both a first hand connection and a second hand connection in my efforts to recruit the elderly male population in the Athens, Georgia community. Along the same lines I believe a more focused recruitment campaign was needed. Upon reflection, fliers around campus and newspaper advertisements, although semi-effective in the short term, do not help build connections for future studies.
Another area that warrants discussion is the image quality of the posterior tibial artery. Subjectively speaking, there was a noticeable difference in image quality between the two groups. Overall, I would say the image quality was much better in the young subjects compared to the old subjects. The reason for this remains a mystery to me. It was as if the constitution of the leg changed with age. Perhaps, the older subjects had more subcutaneous fat or there was slight edema present in some of the older adults that degraded image quality. Maybe the poorer image quality associated with the older group was due to morphological changes that occur in the artery wall with age, although this is all speculation. In order to continue studying the posterior tibial artery in an older population this issue will need to be resolved perhaps by more powerful imaging tools.

Although my results on vasoactive range were inconclusive I feel there is still a need for more research in this area. While not significant at the 0.05 level there was a trend for the older group to have a narrower range compared to the younger group (p=0.095). Considering this was an older population that was healthy it would be interesting to see how vasoactive range behaves in a more diseased population such as diabetics and patients with atherosclerosis are habitual long term smokers.

I was pleased with the pulse wave velocity results. This marks the first time this variable was measured in the Vascular Biology Lab and naturally improvements could be made. Collecting at 300 samples per second, as I did, is the minimum frequency I feel one should collect at. I would recommend collecting at upwards of 500 samples per second in order to accurately and precisely measure the very short time difference between the EKG and pressure waves. Also, I think a more direct measurement of leg pulse wave velocity would be powerful, much more powerful than the somewhat indirect calculated method used in the present study.
CHAPTER FIVE

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APPENDIX A

Baecke Questionnaire of Habitual Physical Activity

1. What is your main occupation?
   never/seldom/sometimes/often/always
   1-2-3-4-5

2. At work I sit
   never/seldom/sometimes/often/always
   1-2-3-4-5

3. At work I stand
   never/seldom/sometimes/often/always
   1-2-3-4-5

4. At work I walk
   never/seldom/sometimes/often/always
   1-2-3-4-5

5. At work I lift heavy loads
   never/seldom/sometimes/often/very often
   1-2-3-4-5

6. After working I am tired
   never/seldom/sometimes/often/very often
   5-4-3-2-1

7. At work I sweat
   never/seldom/sometimes/often/very often
   5-4-3-2-1

8. In comparison with others my own age I think my work is physically
   much heavier/heavier/as heavy/lighter/much lighter
   5-4-3-2-1

9. Do you play sport?
   yes/no
   If yes:
   - which sport do you play most frequently?
     intensity 0.76-1.26-1.76
     - how many hours a week?
       <1/1-2/2-3/3-4/4 Time 0.5-1.5-2.5-3.5-4.5
     - how many months a year?
       <1/1-3/4-6/7-9/9 Proportion 0.04-0.17-0.42-0.67-0.92

   If you play a second sport:
   - which sport do you play most frequently?
     intensity 0.76-1.26-1.76
     - how many hours a week?
       <1/1-2/2-3/3-4/4 Time 0.5-1.5-2.5-3.5-4.5
     - how many months a year?
       <1/1-3/4-6/7-9/9 Proportion 0.04-0.17-0.42-0.67-0.92

10. In comparison with others my own age I think my physical activity during leisure time is
    much more/more/the same/less/much less
    5-4-3-2-1

11. During leisure time I sweat
    never/seldom/sometimes/often/very often
    5-4-3-2-1

12. During leisure time I play sport
    never/seldom/sometimes/often/very often
    1-2-3-4-5

13. During leisure time I watch television
    never/seldom/sometimes/often/very often
    1-2-3-4-5

14. During leisure time I walk
    never/seldom/sometimes/often/very often
    1-2-3-4-5

15. During leisure time I cycle
    never/seldom/sometimes/often/very often
    1-2-3-4-5

16. How many minutes do you walk and/or cycle per day to and from work, school, and shopping?
    <5/5-15/15-30/30-45/45
    1-2-3-4-5