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Research

Noninvasive assessment of vascular function in the posterior tibial artery of healthy humans

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Abstract

Flow-mediated dilation (FMD) measures the ability of an artery to relax in response to increases in blood velocity. FMD, primarily of the brachial artery, has been used as a noninvasive method of assessing vascular health. The purpose of this study was to assess FMD in the lower legs of humans. Six healthy subjects (27 ± 6 yrs) were tested. Doppler ultrasound images of the posterior tibial artery were taken before, during, and after 5 minutes of proximal cuff occlusion. FMD was measured as the percent increase in diameter after cuff release. Vascular tone was calculated using the resting diameter as a percentage of the vessel's vasoactive range. Minimum diameter occurred during ischemia and maximal diameter occurred following reactive hyperemia with local heating. The lower leg was heated with 10 minutes of immersion in 44°C water. Mean diameters at rest, cuff, and during release were 0.267 ± 0.062, 0.162 ± 0.036, 0.302 ± 0.058 cm, respectively. FMD was 13.5 ± 6.6 % and vascular tone was 29 ± 16.3%. We also found that retesting on a second day produced mean diameter values within 8% of the first day. Larger resting diameter (decreased tone) correlated with decreased FMD ($r^2 = 0.73$). These results suggest that FMD and vascular tone can be measured in the posterior tibial artery. This is a potentially powerful tool to non-invasively measure vascular health in the lower legs of people at risk for vascular disease.

Introduction

Cardiovascular disease is the leading cause of death in industrialized nations around the world. Recently, vascular endothelial function has been recognized as an early marker of cardiovascular disease [1]. Flow mediated dilation (FMD) is a non-invasive method that has been used to assess endothelial function [2].

FMD measures the increase in arterial diameter in response to an increase in blood velocity following 5 minutes of cuff ischemia. This technique has traditionally been employed in the brachial artery of the arm. The endothelium, in response to an increase in shear stress, releases nitric oxide (NO) which facilitates relaxation of vascular smooth muscle [2,3]. The brachial FMD test has been noted for its predictive capacity for future cardiovascular complications [4,5]. Over the last decade, research has demonstrated this NO-dependent vasoactive pathway to be impaired with a number of cardiovascular disease risk factors, including advanced age [6], hypertension [7], diabetes [8] and hypercholesterolaemia [2].

A key limitation in the ability of FMD to predict vascular health is that FMD is almost always performed on the brachial artery in the arm [9]. Most exercise interventions prescribed to treat or prevent cardiovascular disease primarily involve use of the legs (walking, cycling, etc.). Consequently, the brachial test may not accurately reflect a person's total body vascular health. Performing FMD in the arm and leg and thereby comparing the two could provide more accurate insight into a person's vascular health [10,11]. In addition to measuring FMD in the legs of healthy subjects, our goal was to measure an index of vascular 'tone'. Vascular tone is defined as the degree of arterial vasoconstriction, and in previous studies it has been suggested that the amount of vasoconstriction will influence the amount of FMD [9].

In order to measure tone, the range of vascular reactivity must first be measured. A previous study [12] measured the range of vascular reactivity of the brachial artery using vessel diameter following cuff ischemia as a minimum and vessel diameter following reactive hyperemia as a maximum. Using a similar protocol, in the leg, we examined the reproducibility of the minimum and maximum diameter measures. We also measured tone, resting diameter or vasoconstriction, expressed as a percentage of the vascular reactivity range and examined its effects on FMD.

Methods

Subjects

Six nonsmoking subjects (4 men and 2 women) participated in the study. Subjects reported no major health problems and had varying activity levels. Informed consent was obtained from the subjects following a detailed description of the experimental procedures. All procedures were approved by the Institutional Review Board at the University of Georgia.

Tests

Two trials were performed on each subject. Each trial was conducted at approximately the same time of day and within 48 hours of each other. Subjects were asked to refrain from eating a high fat meal, smoking, exercising or consuming caffeine prior to each testing session. After 10 minutes of supine rest, a standard flow mediated dilation test was performed on the posterior tibial artery. Immediately following this test, the subject's leg was locally heated for 10 minutes and then a second flow mediated dilation test was performed.

Flow mediated dilation

Doppler images of the posterior tibial artery were recorded every 30 seconds during a 5 minute baseline period. The results from baseline were averaged and used as the initial resting diameter. A pneumatic tourniquet (D.T. Hokinson Inc.) placed around the right leg proximal to the knee was then rapidly inflated (~1 sec) to a pressure 100 mmHg above systolic blood pressure. Images were then recorded every 30 seconds during cuff-inflation for 5 minutes. The pressure was then quickly released from the cuff to induce a reactive hyperemia. Images were recorded every 3–5 seconds for 2 minutes to determine maximum diameter. The highest diameter reading was used in the calculation of FMD. Images were then collected every 15 seconds for an additional 3 minutes to monitor the return of arterial diameter to resting values. Figure 1 provides an illustration of the resting, cuff, and cuff release periods.

Measurement of vessel diameter

The diameter of the posterior tibial artery was measured using high-resolution B-mode imaging (400 CL, GE Medical) with a 9–13 MHz linear-array ultrasound transducer. The leg of each subject was marked, to insure probe placement was not different from one testing session to the next. Magnification and focal zone settings were adjusted to optimize imaging of the vessel walls. Gain was held constant throughout. All diameter measurements were taken at the end of diastole. End diastole was identified visually by observing the variation in vessel diameter with each heartbeat. Artery diameters were measured, following each test, using semi-automated edge-detection software specially coded for use with Labview (National Instruments). A region near each vessel wall was identified visually, and manually marked on the image. The program then automatically located the vessel wall by analyzing pixel intensity, and finding the area of greatest pixel contrast. This procedure was performed for both walls and then a parallel line was automatically drawn between the walls in order to measure vessel diameter.

Local heating

Local heating of the lower leg was used to increase blood velocity through the posterior tibial artery. Warming of the skin is thought to increase blood flow locally in the absence of systemic autonomic influence [13–18]. Each subject's leg, up to the knee, was placed in 44°C water for 10 minutes. The subject was then removed from the warm water and positioned on an examination table. A heating pad was then wrapped around the subject's foot and a second heating pad was placed under the subject's calf. The heating pads were used to maintain leg temperature while a second FMD test was performed.

Calculations

Flow mediated dilation was calculated as the percent change in posterior tibial artery diameter from baseline to peak following reactive hyperemia (%FMD). Baseline diameter (BD) was calculated as the average of all images taken during the 5-minute baseline period. Results recorded during the last two minutes of tourniquet inflation were averaged and used as an index of minimum diameter (MD). The largest measured vessel diameter after cuff release was used as the peak diameter (PD). Vascular tone was calculated as baseline diameter as a percentage of the total diameter range [(PD-BD)/(PD-MD) *100]. Day to day variability was performed on 4 of 6 subjects (3 men, 1 woman). Absolute diameter differences for each subject were calculated during all conditions between the two testing days. These differences were then averaged across

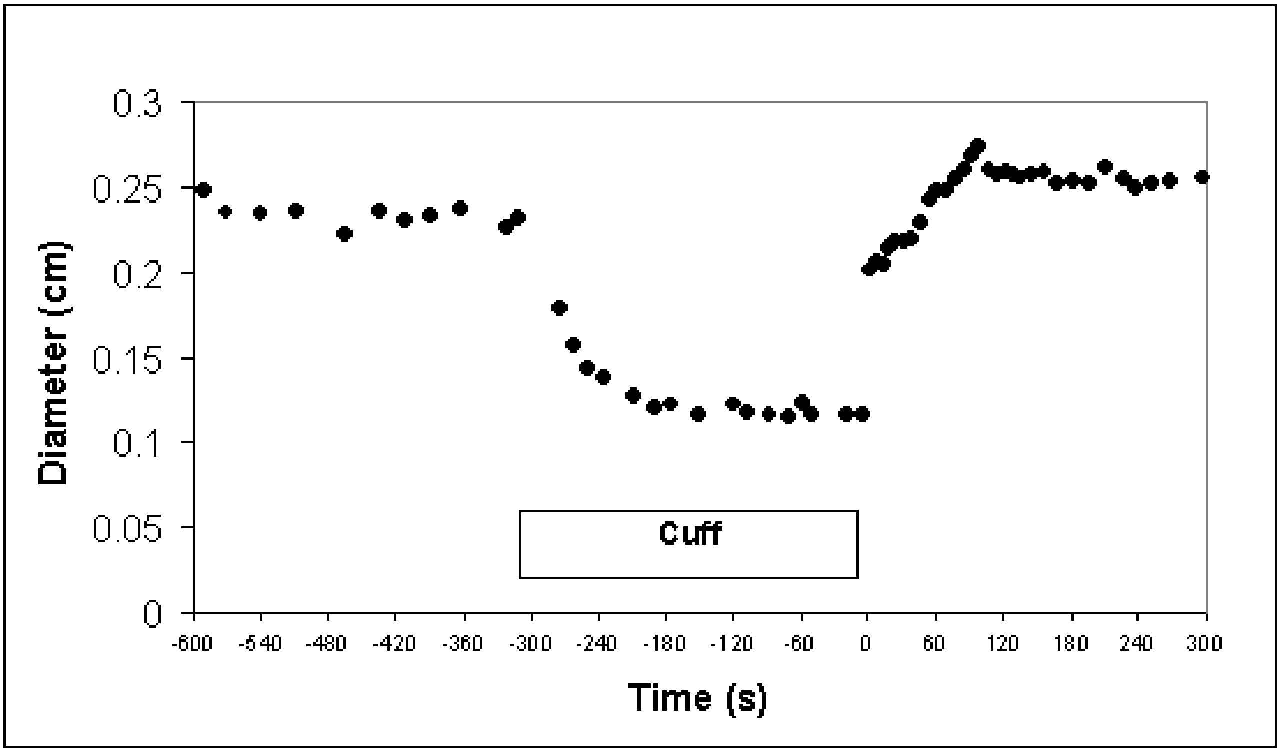

Figure 1
Representative example of the flow mediated dilation protocol and accompanying changes in arterial diameter in the lower leg. Cuff ischemia was proximal to the artery resulting in ischemia and reduced diameter. Reactive hyperemia upon cuff release increased blood velocity (not shown) and diameter.

all subjects and a percent difference from day 1 values was calculated.

Results

Results are mean ± SD. The six subjects had a mean height of 182.5 ± 6.3 cm, mean weight of 79.4 ± 11.3 kg, and mean age of 27.8 ± 6.8 years. A representative graph of arterial diameter over time is presented in Figure 1. Of particular note is the fact that minimum diameter occurred following 2 to 3 minutes of cuff ischemia. The diameter plateaued during the remainder of the cuff period. This was observed in all subjects. Peak diameter occurred 1 to 2 minutes following cuff release in all subjects (98 ± 37 sec.). Mean posterior tibial artery diameters before, during, and after cuff ischemia for the six subjects can be seen in Figure 2. Mean FMD for the subjects was 14.3 ± 6.8% while resting vascular tone was 29 ± 16.3 %.

Absolute day-to-day variations in vessel diameter were 6.07%, 18.4%, and 4.05% between days for resting, cuff, and cuff release. The resting and cuff release diameters had comparable to day-to-day variability to values measured in our lab in the brachial artery (unpublished results) and in other previous studies [19]. This suggests that our methods, if applied to a larger population, are reproducible or at least as reproducible as other FMD measures.

Resting vascular tone showed a strong correlation with FMD ($r^2 = 0.73$) (Figure 3). This correlation was better than the correlation between FMD and absolute resting diameter (Figure 4).

Discussion

The purpose of this study was to determine the feasibility of measuring flow mediated dilation (FMD) in the posterior tibial artery of the leg. FMD has been widely measured in the brachial artery of the arm [9], but few if any studies report FMD in the leg [10,11]. Of those studies one reports FMD in the femoral artery [20], while the other reports values from the posterior tibial artery of subjects with coronary heart disease [11]. Our FMD values from the posterior tibial artery were larger than the values re-

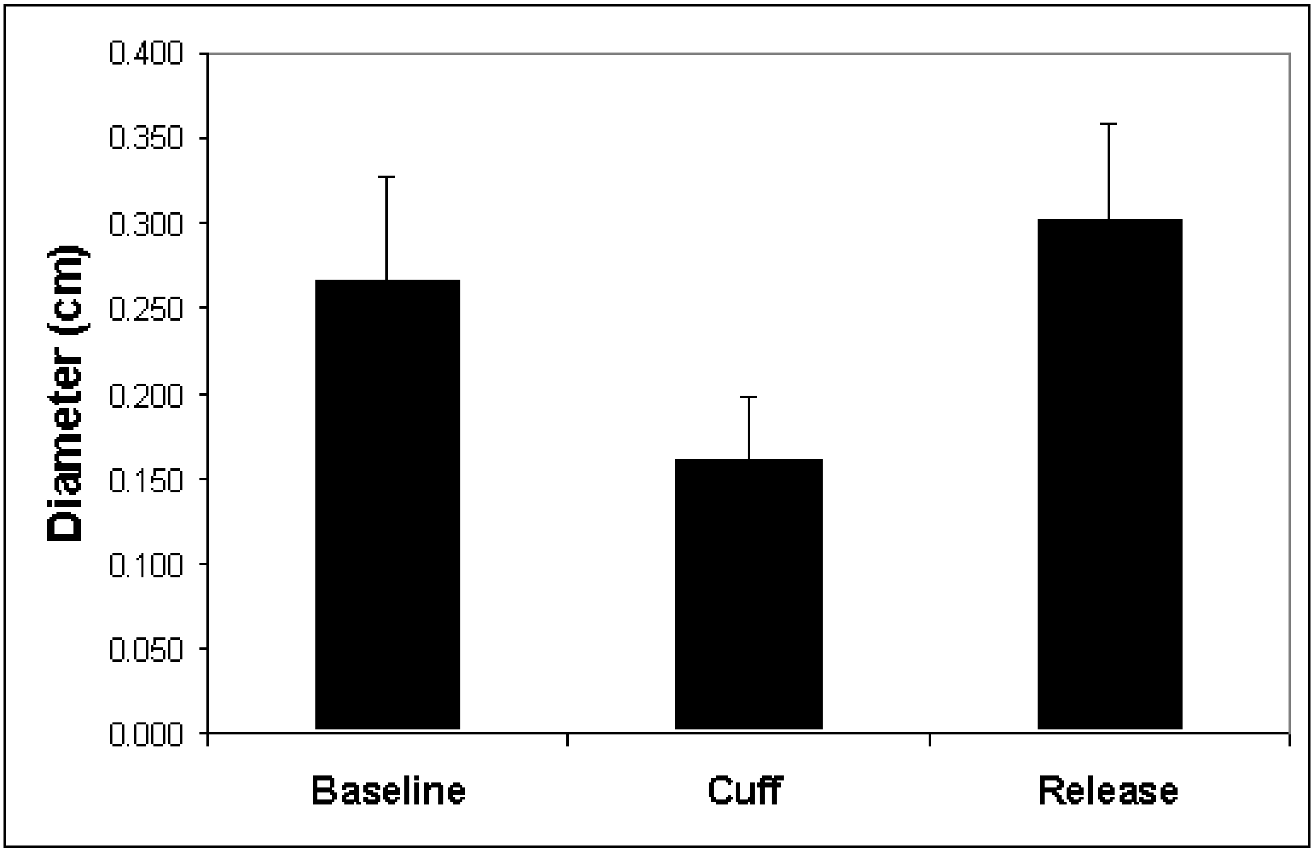

Figure 2
Day 1, average posterior tibial artery diameters at rest, during cuff and following cuff release. Values are mean ± SD for. Increase following cuff release was 14.3%.

ported by *Gokce et al.* (14.3 ± 6.8% vs. 9.7 ± 2.1%) [11]. A possible explanation for our larger values was our use of young healthy subjects, who would be expected to have larger values when compared to older, diseased subjects. Our FMD values were also slightly larger than FMD values from similar subjects measured by our lab in the arm 11–12 ± 4% (unpublished results). In addition our values were slightly larger than those reported in the literature for healthy young subjects in the arms [2,21]. It is possible, given its smaller size and location in the leg, that the posterior tibial artery has a greater vascular reactivity than the brachial artery.

The day-to-day variability our measure of FMD in the leg was similar to values reported in the arm and leg. Our values of 6% and 4.05% for absolute day-to-day percentage differences for resting and maximal diameter are similar to our data from the arm 3.39–6.72% (unpublished results) and from previous studies [19]. *Gokce et al.* found, although not reported in this manner, absolute percentage diameter differences of approximately 5.8% from day-to-day in the posterior tibial artery which is consistent with our data. Our value of an 18.4% difference during the cuff, is considerably larger than our values from rest and during cuff release. It is likely that this difference is do to the increased difficulty of imaging the vessel during this period. Also, since the vessel is smaller during this period, any day-to-day differences will represent a greater percentage of the overall vessel diameter. This suggests that our measure of FMD in the leg, while variable, is as reproducible as FMD measured in the arm and leg by others. As with FMD measured in the arm, variations in %FMD measured from day-to-day were observed (14.3% on day 1 vs. 18.7% on day 2). This represents a 31% difference from day 1 to day 2. Although this number appears to be rather large, it serves to illustrate the manner in which small day-to-day changes in vessel diameter can have large effects on %FMD. Although not reported in this manner, a previous study of FMD in the posterior tibial artery found similar results [11]. The large day-to-day variability

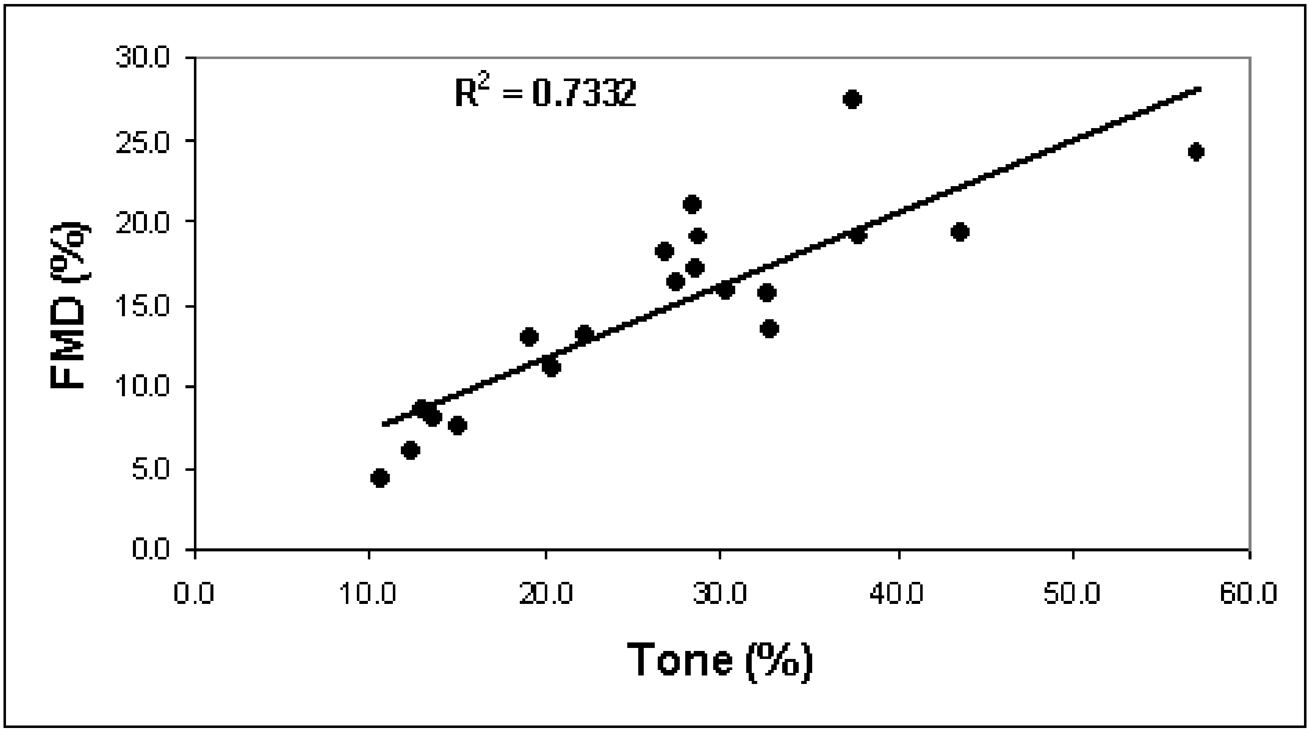

Figure 3
Relationship between %FMD and % resting vascular tone. FMD is increase above baseline following cuff release, and tone represents resting diameter in relation to maximum diameter. Data points represent values from room temperature as well as heated conditions for six subjects on day 1 and for four subjects on day 2 (20 total). R^2 value of .73 demonstrates the positive relationship between FMD and tone. As tone increases, resting diameter will decrease thus allowing for a greater FMD.

in the measurement of FMD, which can be attributed to both the small size of the vessels as well as the difficult nature of the technique, is a limitation of the method.

One method of improving the variability of FMD is to correct the values for daily variations in vascular tone. This study used a novel approach to non-invasively assess vascular tone. Previous studies have shown strong relationships between resting diameter and the magnitude of FMD [9]. However, studies using resting diameter were performed on subjects who were homogeneous in body size. A heterogeneous population was used in our study, and consequently subjects with different body sizes were used. Clearly larger subjects will have larger arteries, but will have the same relative amount of FMD (all else being equal). Thus resting diameter must be normalized to correct for body size. We feel our vascular tone measure meets this criterion. Based on previous research [12–18] and our own observations, tone was calculated based upon a minimum diameter taken during cuff occlusion and a maximal diameter taken after heated cuff release. This produced a strong relationship between FMD and resting tone ($r^2 = 0.73$), which exceeds values reported in previous studies examining the relationship between FMD and resting diameter [9] and our own relationship between FMD and resting diameter ($r^2 = .57$).

While future studies are needed for confirmation, we agree with a previous study [12] in that this protocol can elucidate a valid minimal diameter. It represents a physiological response of the vessel to decreased intramural pressure (proximal cuff). The diameter values during cuff inflation leveled off after 2–3 minutes, thus suggesting a stable measure. The maximum diameter was more difficult to measure. We chose local direct heating and proximal cuff ischemia to induce a maximal diameter. This was based on previous studies in the arm where increases in brachial artery diameter under similar conditions where FMD reached 18% (unpublished results), similar to values reported for maximal pharmacological diameter changes [9].

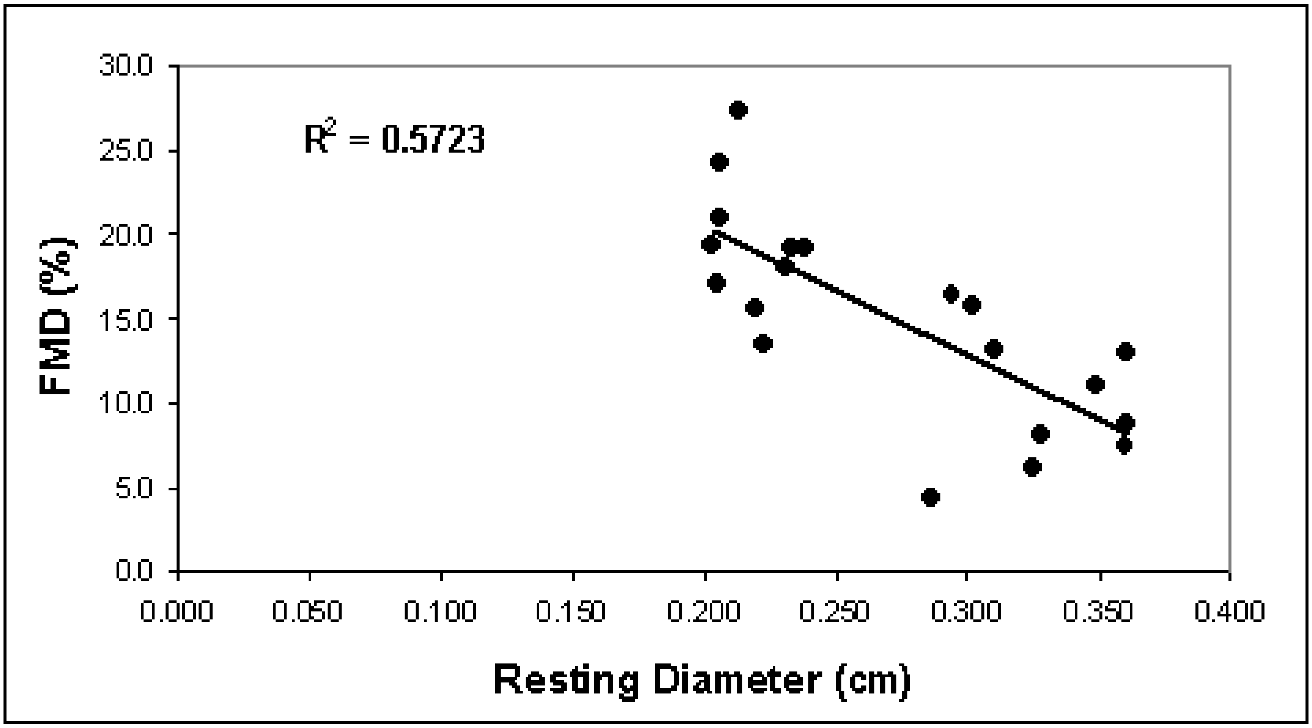

Figure 4
Relationship between %FMD and resting vessel diameter. FMD is increase above baseline following cuff release, and resting diameter is an average of values taken over 5 minutes. Data points represent values from room temperature as well as heated conditions for six subjects on day 1 and for four subjects on day 2 (20 total). R^2 value of 0.57, with a negative slope demonstrates the inverse relationship between FMD and resting diameter

A key to the importance of this study is the relationship between FMD and endothelial function. Numerous studies have used FMD as an early marker of vascular health [1,9]. However, a recent study has reported a lack of correlation between FMD measured in the arm and invasive pharmacological measures of endothelial function [22]. It is not clear how to interpret the lack of correlation between two measures designed to evaluate the same biochemical pathways. FMD has a clear advantage in human studies because of its noninvasive nature. A correction for resting vascular tone, as in our experiment, could result in better agreement between these two measures.

In conclusion, our findings suggest that FMD measurements in the posterior tibial artery of the leg can be made with accuracy and reproducibility similar to FMD measurements made in the brachial artery. These measures, when combined with a measure of vascular tone, may provide a better and more accurate picture of endothelial function and vascular health. Flow mediated dilation in the legs may also provide a better indicator of total body vascular health as well as providing a more accurate picture of the effects of an intervention (walking, cycling, etc.) on endothelial function. The results of our measurements of vascular tone should be considered to be preliminary, as future studies will be needed to confirm or improve the methods of measuring minimum and maximum diameters.

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