THE RELATIONSHIP BETWEEN BLOOD VELOCITY AND CONDUIT ARTERY VASOACTIVITY: ROLE OF NITRIC OXIDE?

by

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(Under the Direction of Kevin McCully)

ABSTRACT

This study had three major purposes: 1) investigate the blood velocity-vasoactivity relationship; 2) determine whether indirect local heating can be used in conjunction with flow mediate dilation (FMD); 3) determine the effects of cigarette smoking on the blood velocity-vasoreactivity relationship. Blood velocity through the brachial artery was manipulated through the use of: 1) ischemia; 2) handgrip exercise; and 3) indirect local heating. Additionally, at the highest level of indirect local heating (42°C) FMD was repeated (%42°C FMD). A strong and reproducible relationship was observed between time average maximum blood velocity (Tamax) and brachial diameter independent of the method used to increase blood velocity (R^2=0.92, P >0.05). A novel finding was a greater effect size for 42°C %FMD compared to room temperature %FMD (%FMD) (11.9$\pm$5.1 %FMD vs 18.4$\pm$10.4 42°C %FMD; P.<0.05). However, whilst smoking one cigarette impaired %FMD, %42°C FMD was not attenuated, and there was no effect seen on the Tamax-diameter relationship.

INDEX WORDS: Blood velocity, vasoactivity, flow mediated dilatation, nitric oxide, smoking; indirect local heating, handgrip exercise.

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DEDICATION

I would like to start by thanking my advisor, Dr Kevin McCully. I thank him for giving me a chance in the first place to study at UGA, but more importantly for encouraging my scientific curiosity and the all the hard work he has put in to setting me on the right road as not just a scientist, but a scientist who is able to develop and communicate his ideas. I look back and feel proud when I realize the progression I have made since joining the team here thanks to his help.

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Most of all I would like to thank my grandparents Kath and Dave. Without them I would not be here. They have given me so much support, both through encouragement and financial aid. I cannot express how much their unreserved love means to me so hopefully these words will let them know I appreciate them, if not just how much.

Finally, I would like to mention someone special. I am moving to the next stage of academia and my life with my heart smiling. I could not be happier with life. I love you Danielle, and I think fate loves us!

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The endothelium lining vascular arteries has garnered much attention over the last decade; the attraction growing with each new study associating endothelial dysfunction with increased risk for contracting cardiovascular diseases (CVD) (Accini et al, 2001; Celermajer et al, 1992; Clarkson et al, 1996; Herrington et al, 2001; Iayama et al, 1996; Murakami & Arai, 2001; Poredos et al, 1999; Pepine et al, 1997; Schroeder et al, 1999). In light of these findings investigators have implemented a number of invasive and non-invasive experimental models to increase the understanding of endothelial function *in vivo* in humans. The most prominent non-invasive test for screening endothelial dysfunction is flow-mediated dilation (FMD) of the brachial artery (Celermajer et al, 1992). This measure has been associated with a comprehensive range of CVD risk factors. However, the sensitivity and reproducibility of the current FMD methodology has been brought into question (Hijmering et al, 2001). The current study aims to improve the sensitivity and reproducibility of the brachial FMD methodology.

The endothelium.

The endothelium is a flat mono-layer of cells that cover vascular lumina throughout the body. Functionally, the endothelium is a large autocrine, pancrine, and endocrine organ that plays a key role in vascular homeostasis (Luscher & Barton, 1997). In particular, the endothelium has been recognized for the important role it plays in

regulating vascular reactivity via the release of dilator mediators, including nitric oxide (NO) (Moncada & Higgs, 1993) and prostaglandins (Koller et al, 1993). Of these dilator mediators NO is the primary compound responsible for dilating arteries and in turn regulating vascular reactivity (Moncada & Higgs, 1993). NO is a gas that is continuously released from the endothelium where it is synthesized from the precursor L-arginine in a reaction catalyzed by nitric oxide synthase (eNOS isoform in endothelium) (Baron *et al*, 1999). Besides its role in regulating vascular reactivity NO inhibits platelet aggregation (Moncada & Higgs, 1993), modulates leukocyte-endothelium interactions by altering cell adhesion molecule expression and reducing monocyte adherence (Tsao et al, 1996), and inhibits the proliferation of smooth muscle.

There is now a consensus of opinion that the endothelium is the central site defining vascular dysfunction in the CVD state (Pepine et al, 1997). Research findings over the last decade have recognized this pathway to be impaired by all CVD risk factors, including advanced age (Herrington et al, 2001), hypertension (Iayama et al, 1996), diabetes (Clarkson et al, 1996), and hypercholesterolaemia (Celermajer et al, 1992). Recently it has also been demonstrated that impaired FMD responses occur in young children with low physical activity levels (Abbott et al, 2002). This suggests the initiation of CVD to onset at an early age.

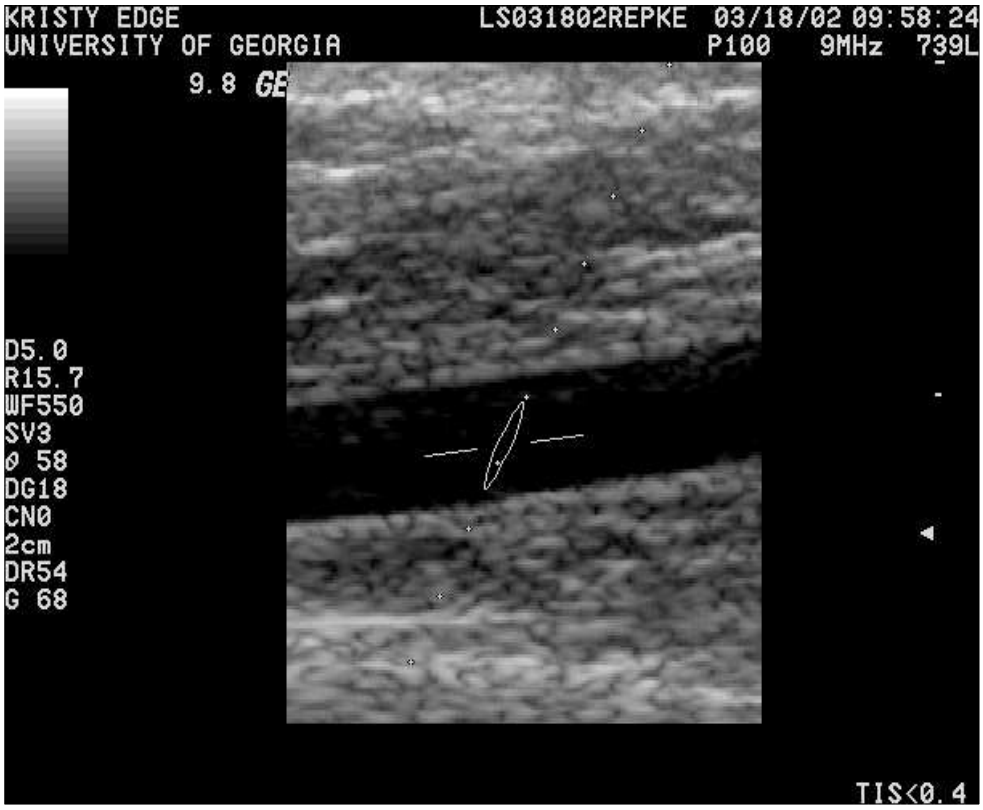
Environmental CVD risk factors have also been demonstrated to impair this pathway, most notably diets high in fat and smoking. However, the relationship between dietary intake and endothelial function has proved to be complex, and thus far is unresolved (Nurminen et al, 1998), though smoking clearly has acute as well as transient effects on endothelial function (Poredos et al, 1999).

The assessment of vascular endothelial health can serve as sensitive marker for screening and monitoring cardiovascular health and assessing long-term as well as short-term risk factors.

Assessing endothelial health.

The duplex Doppler has been a technology widely used to study endothelial function of the brachial artery as a proven surrogate for both peripheral as well as cardiovascular health (Anderson et al, 1995; Neunteufl et al, 1997) (see figure 1.1). Endothelium-dependant agonists such as acetylcholine can be used to induce an endothelial response (Furchgott & Zawadzki, 1980), but such practice is invasive and often unpractical, especially for use within clinical settings. Alternatively, a method which is *non-invasive* entails increasing blood-velocity shear stress through increasing blood flow demand downstream from the site of vessel imaging. Typically a pneumatic tourniquet will be placed around the forearm just below the elbow and inflated to a super-systolic blood pressure for 5 minutes. When the occlusion is released the increased blood flow demand downstream (reactive hyperemia) will increase blood velocity through the artery upstream, and cause vasodilation of the artery by increasing shear stress (see figures 1.2 & 1.3). This response has been termed flow-mediated dilation (FMD) (Celermajer et al, 1992) and is typically expressed as the percentage increase in the artery diameter from baseline to the peak vasoactive (diameter) response following reactive hypermia. This test has been noted for its predictive capacity for future cardiovascular complications (Murakami & Arai, 2001; Schroeder et al, 1999).

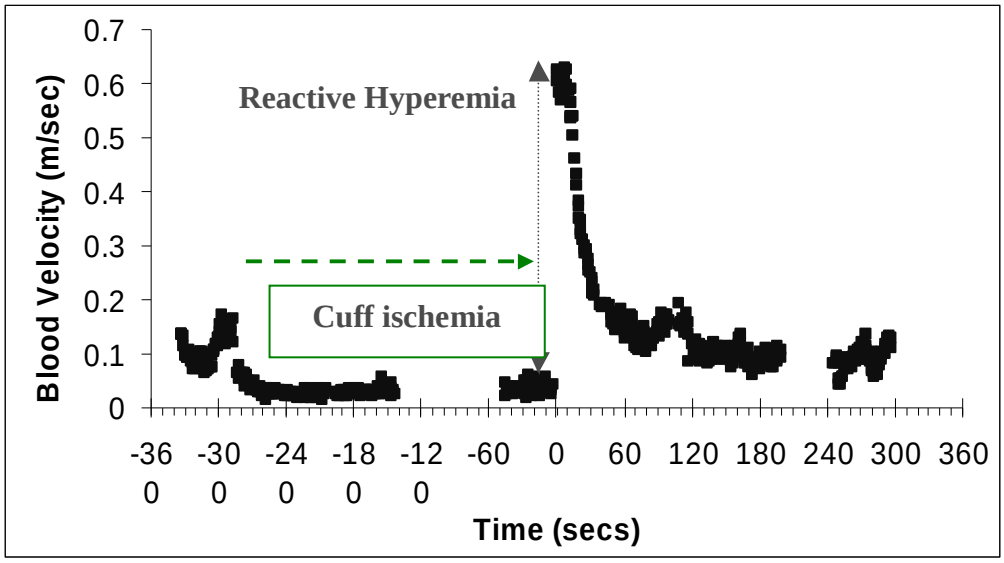
Figure 1.1.. B-Mode ultrasound image of brachial artery



Recently, the Colombain study to assess the use of noninvasive determination of endothelium-mediated vasodilation (CANDEV) (Accini et al, 2001) was conducted to identify a set of normal %FMD values. From a population of 253 normotensive healthy volunteers with no cardiovascular risk factors, a mean %FMD of 14% was found, in contrast to a mean of only 7% in those with a least one risk factor. A 10.4 %FMD cut-point was identified to classify subjects with at least one risk factor. Using this cut-off point, endothelial dysfunction was three times more frequent in subjects with than in subjects without cardiovascular risk factors. Of note, obesity, hypecholesterolemia and

smoking were the modifiable risk factors with the largest independent significant negative effects on %FMD.

Figure 1.2. Reactive hyperemia following 5 minutes ischemia,. measured from the brachial artery of a young healthy female subject.

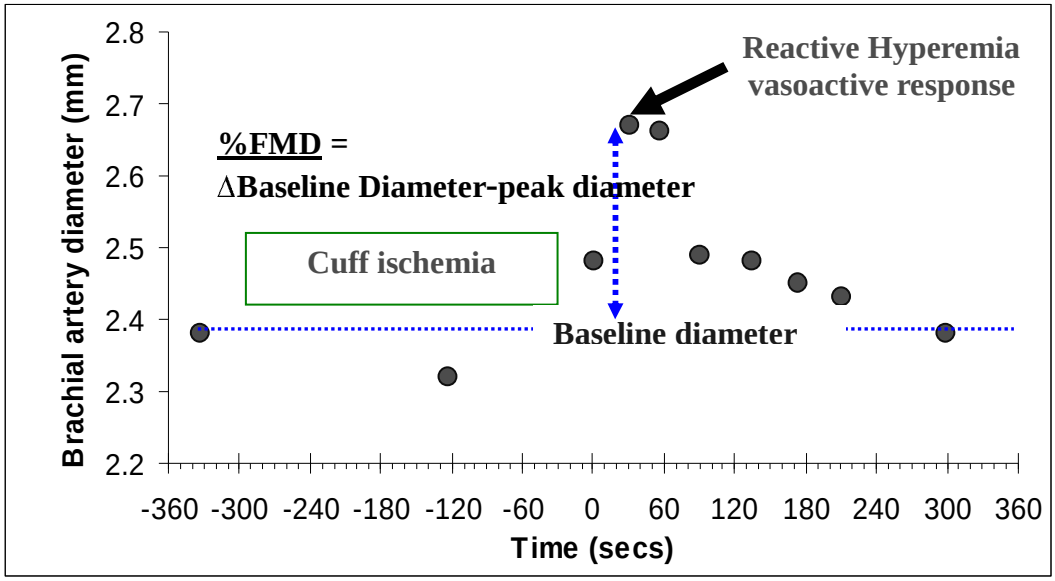


Note: See figure 3 for vasoactive response to reactive hyperemia.

FMD clearly becomes impaired with certain disease states, including: hypertension (Celermajer et al, 1992), atherosclerosis (Iayama et al, 1996), and insulin resistant (Baron et al, 1999). Disease states have also been found to However, the effect size (i.e. the decrease in %FMD) is small and this equates to large sample sizes being required for a significant effect. It is questionable whether or not FMD can sensitively distinguish between diseased population groups, or be used to screen populations with CVD risk factors before the onset of disease. It is also unlikely that FMD is able to determine inter-individual differences amongst populations. Thus, whilst FMD has potential to sensitively

assess CVD risk, a more sensitive test is required before this methodology can be clinically employed to fulfill this potential.

Figure 1.3. Vasoactive response to increased blood velocity following 5 minutes of ischemia.



Note: Expanded scale; diameter change is from 2.2mm to 2.8mm.

FMD and shear stress

It has been proposed that vascular tone is regulated in response to stimuli generated through the velocity profile imposed on the arterial wall. There is evidence that acute induction of low blood velocity constricts the brachial artery (Stadler et al, 1998). Similarly, increased blood velocity provokes a vasoactive response (Celermajer et al, 1992), i.e., post ischemia induced via a pneumatic tourniquet – through the release of dilator mediators, most notably NO (Moncada & Higgs, 1993). This blood velocity profile vasoactive stimuli has been coined 'shear stress'. However, the peak vasoactive response

is seen some time (45 to 90 seconds) after reactive hyperemia, and as such it is still unclear how the velocity profile creates this 'shear stress'. No study has manipulated blood velocity profile *in vivo* and simultaneously measured the vasoactive response. Further study is needed to better identify the relationship between the velocity profile and endothelium dependant vascular reactivity.

Exercise

Studies looking at the relationship between exercise and endothelium dependant vasoreactivity have produced conflicting results. Some investigators have found the endothelium not to play an active, or at least a substantial, role during exercise (Frandsenn, 2001; Radegran & Hellsten, 2000). However, others have suggested that vasoreactivity may actually be dependent on the endothelium during exercise (Katz et al, 1996). Some studies may not have found endothelium-dependant vasoreactivity during exercise because: 1) the site of interest to the investigator was proximal to active muscle(s) and therefore directly influenced by exercise induced metabolites; and 2) the exercise activity and intensity chosen was such that marked systemic responses were expected. Investigators have not studied the importance of the endothelium distal to the active muscles during exercise, where it is not being actively influenced by metabolites produced from the active muscle(s). Therefore, not only is the exact role of the endothelium to vasoreactivity unclear during exercise, it is less clear what effect exercise has on endothelium dependant vasoreactivity in arteries that are distal to active muscle(s). The current study will evaluate the use of low to moderate intensity exercise of a small muscle group to induce changes in the blood velocity distal to active muscle(s).

Local heating

Local warming of the skin induces localized vasoreactivity that is graded with skin temperature, with the maximal vasoreactivity and blood velocity response occurring at 42°C (Johnson et al, 1986; Taylor et al, 1994). The mechanism responsible for this response is not fully understood, but endothelial NO production is thought to play a central role (Kellogg et al, 1998; Kellogg et al, 1999; Shastry et al, 1998). There is evidence to suggest that that this response may be produced through a neurogenic reflex with NO serving a permissive role to some unknown neurotransmitter (Crandall & MacLean, 2001).

Local heating can be used to *indirectly* increase blood velocity through the artery upstream from the area of heating. The current study hypothesizes that this increase in blood velocity upstream is due to increased shear stress since systematic influence is thought to be non-evident or at least marginal in effect. Of note, *indirect local-heating* may be of particular interest because it can be used to increase local blood velocity for a controlled period of time.

Indirect local heating also has the potential to increase the reactive hyperemia response to ischemia induced via occlusion, and consequentially to increase the vasoactive response to increased blood-velocity post-occlusion. More importantly, indirect local heating can potentially be used as a non-invasive, sensitive, and controllable means of inducing an upstream local endothelial response. The current study specifically evaluated whether indirect local heating can: 1) be used in conjunction with FMD to provide a more sensitive and reproducible means of assessing endothelial health when compared to the

standard FMD test; and 2) be used unaccompanied to control upstream vasoreactivity as a means of assessing vascular health.

This study is comprised of two parts. This study controlled blood velocity through the brachial artery by increasing downstream blood flow demand through the use of progressively increasing exercise intensity. The blood velocity response to exercise was compared to that induced through progressive indirect local heating. Low to moderate intensity exercise of a small muscle group has been chosen in order not to induce systemic responses. At the highest level of indirect local-heating (~42°C) the brachial FMD test was administered, i.e. occlusion for 5 minutes, followed by 5 minutes rest to determine if heating influences the sensitivity of reactive hyperemia and hence vasoreactivity. These responses were compared to those determined using the standard FMD test. Reproducibility was determined by repeating each stage of testing on one subsequent day under the same conditions. Blood pressure and heart rate were continuously monitored to determine whether or not the exercise or local heating protocols provoked systemic responses. To determine the role of the endothelium a population of smokers were tested before and after smoking one cigarette.

Purpose Statement

The purpose of this study is to investigate the relationship between blood velocity and the vasoactive response of the brachial artery. A better understanding of the relationship between blood velocity and the vasoactive response can potentially be used to develop a sensitive test of vascular endothelial health. This study will also determine whether cigarette smoking attenuates the vasoactive response to increased blood velocity.

An additional purpose of this study will be to determine whether local indirect heating can be used in conjunction with FMD to improve the sensitivity and reproducibility of the current FMD test.

Questions:

1. What is the relationship between blood through the brachial artery and the vasoactive response of the brachial artery?
2. Do different methods of increasing blood velocity produce the same vasoactive effects?
3. Can better understanding of the relationship between the blood velocity and the vasoactive response be used to develop a more sensitive test of vascular health?
4. How does cigarette smoking influence the relationship between blood velocity and the vasoactive response?

Specific study hypothesis:

1. Brachial flow-mediated dilation is a reproducible vascular health test, i.e. <5% variation between tests day-to-day
2. There is a linear relationship between blood velocity through the brachial artery and the diameter of the brachial artery, independent of the method used to increase blood velocity
3. Smoking alters the blood velocity-diameter relationship by reducing the slope of the linear relationship
4. FMD plus local indirect dilation induces a greater vasoactive response than FMD alone, i.e., FMD plus local indirect heating produces a greater effect size than FMD at room temperature
5. FMD plus local indirect heating is more reproducible than FMD at room temperature

Significance of the Study

CVD is a worldwide epidemic (Baric, 1999; Corti *et al*, 1996). The vascular endothelium is thought to be integral to the progression of the cardiovascular diseased state. There is a demonstratable relationship between endothelial health and the ability of the endothelium to vasoreact in response to local stimuli such increased blood velocity. However, current non-invasive flow-mediated dilatation methodology for monitoring vascular endothelial health may lack sensitivity and reproducibility. The purpose of the current study is to develop a sensitive and reproducible means of assessing and

monitoring endothelial health. The current study increases understanding of the relationship between blood velocity and vasoreactivity, and the importance of endothelium derived nitric oxide to this relationship.

CHAPTER 2

THE RELATIONSHIP BETWEEN BLOOD VELOCITY AND CONDUIT ARTERY VASOREACTIVITY: ROLE OF NITRIC OXIDE?[1]

[1]Lee Stoner, Kevin McCully, and Kristy Edge. To be submitted to the Journal of Applied Physiology

Abstract

The vascular endothelium is integral to the development of the cardiovascular diseased (CVD) state. Endothelial health can be predicted using brachial flow mediated dilatation (FMD), a measure of the capacity of the endothelium to vasoact in response to increased blood velocity shear stress. However, FMD is subject to a number of limitations and may lack sensitivity and reproducibility. This study has 3 major purposes: 1) to investigate the relationship between blood velocity and vasoactivity; 2) determine whether indirect local heating can be used in conjunction with FMD to improve the sensitivity and reliability of FMD; 3) determine the effects of cigarette smoking on the blood velocity-vasoreactivity relationship. Six male and female non-smokers (23±3 years) and an additional six male and female (20±1 years) occasional cigarette smokers were recruited. Blood velocity through the brachial artery was controlled through the use of: 1) ischemia; 2) progressive intensity handgrip exercise; and 3) progressive local indirect heating. At the highest level of indirect heating (~42°C) FMD was repeated to determine if heating influences the sensitivity of the vasoactive response. Reproducibility was determined by repeating the study conditions on one subsequent day. To determine the effects of cigarette smoking, the smokers were subjected to the above procedures before and after smoking one cigarette on subsequent days. We found a strong and reproducible relationship between time average maximum blood velocity (Tamax) and the vasoactive response independent of the method used to increase blood velocity (R^2=.92, P >.05). A novel finding is a greater effect size and reproducibility for 42°C FMD compared to room temperature FMD (11.9±5.1 vs 18.4±10.4 %FMD). However, whilst smoking 1 cigarette impaired the room temperature FMD response, 42°C FMD was not attenuated, and did not affect

vascular reactivity in healthy young subjects. In conclusion, handgrip exercise and indirect local-heating have potential as vascular health screening tools when combined with the standard FMD test. 42°C FMD increases the FMD effect size, though further investigation is warranted regarding mechanistic implications of indirect local-heating.

Introduction

There is now a consensus of opinion that the endothelium is integral to the development of CVD (Pepine et al, 1997). Endothelial health can be assessed *non-invasively* using brachial flow mediated dilatation (FMD) (Celermajer et al, 1992). This test monitors the ability of the endothelium to relax vascular smooth muscle in response to increased blood-flow shear stress. The primary mechanism thought responsible for this vasoactive response is the local release of nitric oxide (NO) from the vascular endothelium (Celermajer et al, 1992; Moncada & Higgs, 1993) in response to an increase in shear stress.

The brachial FMD test has been noted for its predictive capacity for future cardiovascular complications (Murakami & Arai, 2001; Schroeder et al, 1999). Indeed, research findings over the last decade have recognized this NO-dependent vasoactive pathway to be impaired by all CVD risk factors, including advanced age (Herrington et al, 2001), hypertension (Iayama et al, 1996), diabetes (Clarkson et al, 1996) and hypercholesterolaemia (Celermajer et al, 1992).

Environmental CVD risk factors also impair this pathway, most notably the consumption of diets high in fat and cigarette smoking have been noted as prominent though potentially reversible risk factors (Poredos et al, 1999). Smoking in particular has potent acute as well as transient endothelial function implications (Poredos et al, 1999).

Exactly how inherent and environmental risk factors interact to impair endothelial function and the release of NO is not clearly understood, but such findings do highlight the potential for gauging vascular endothelial health as a surrogate marker for screening and monitoring cardiovascular health and identifying long-term as well as short-term risk factors.

The mechanism responsible for the release of vascular endothelial NO is thought to be entirely local and due to stimuli initiated from the blood velocity profile, i.e. sheer stress. However, the peak vasoactive response is seen some time (45 to 90s) after reactive hyperemia, and as such, it is still unclear how the blood velocity profile creates this 'shear stress'. Further study is needed to better identify the relationship between blood velocity and the endothelium-dependent vasoactive response *in vivo*.

The current study used: 1) reactive hyperemia; 2) progressive intensity handgrip exercise; and 3) indirect local-heating to manipulate blood velocity *in vivo* to better understand the relationship between blood velocity and the vasoactive response.

This study used indirect local-heating for an additional purpose**.** Warming of the skin is thought to increase blood flow locally without systemic autonomic influence (Crandall & MacLean, 2001; Johnson et al, 1986; Kellogg et al, 1998; Kellogg et al, 1999; Shastry et al, 1998; Taylor et al, 1994). Of particular interest to the current study, local heating can potentially be used to *indirectly* increase blood velocity through an artery upstream from the area of heating without confounding systemic effects.

This study used *indirect local-heating* in conjunction with FMD (42°C FMD). Indirect local heating has the potential to 1) increase the reactive hyperemic response to ischemia and therefore increase the vasoactive response; and 2) provide a more sensitive and reproducible FMD test due to a more consistent vasoactive response

Materials and Methods

Subjects and study design.

A total of 12 healthy and moderately active subjects were recruited for this study. Informed consent was obtained from the subjects after they were given a detailed description of the procedures. The study was approved by the University of Georgia Institutional Review Board.

6 healthy male and female non-smokers (20 to 26 years old), and 6 otherwise healthy male and female (19 to 23 years old) occasional cigarette smokers (reported to smoking $\leq$1 Packet per week) were required to make two visits to the laboratory. The 6 non-smokers were used to test the reproducibility of the study measures (reproducibility group), and the 6 smokers were used to test the implications of cigarette smoking on these measures (smoke group).The reproducibility group were subjects to two identical trials over two days at the same time of day. The smoke group were also tested on two separate days: a control trial (i.e. non-smoking) and a smoking trial, i.e., repeating the control trial study protocols after smoking 1 cigarette on a subsequent day, and at the same time of day. The second testing session was completed within 48 hours of the first session for both studies. All testing sessions were performed on weekdays between the hours of 7-10am. The group of smokers verified that they did not smoke for seven days

prior to reporting to the laboratory for any stage of testing. All subjects were asked to report to the laboratory in the fasted condition, having refrained from exercise for 48 hours prior to testing. Subjects were also asked not to consume caffeine or administer any medications with know vascular complications prior to testing. All stages of testing were performed in a climate controlled laboratory setting. Subjects were excluded from the study if they demonstrated any CVD health risks.

This study manipulated blood velocity through the brachial artery by increasing downstream blood flow demand through the use of: 1) ischemia; 2) progressive intensity handgrip exercise; and 3) indirect local-heating. Low to moderate intensity handgrip exercise of a small muscle group was chosen in order not to induce systemic autonomic responses. A water bath was heated to ~40°C and then ~42°C to heat the forearm and increase blood velocity through the upstream brachial artery. At the highest level of indirect heating (~42°C) FMD was repeated to determine if indirect local heating influences the reliability of the vasoactive response. Reproducibility was then determined by repeating each stage of testing with the reproducibility group on one subsequent day (Trial 2) under the same conditions. To determine the role of the endothelium, the 6 smokers were subjected to the above protocols before (control trial) and after smoking (smoke trial) one filtered cigarette (12 mg tar, 1.0mg nicotine**).** Blood pressure and heart rate were continuously monitored to determine whether or not local heating provoked systematic autonomic responses. Skin temperature recordings were taken to indicate any direct heating effects at the site of the imaged artery.

Measurement of blood velocities and vessel diameter.

A GE 400CL duplex color Doppler imager (GE Medical) was used to assess FMD in the brachial artery, along with simultaneous measurement of the subjects blood velocity profile. All brachial artery diameter and blood velocity measurements were taken in the left arm for all stages of testing. Blood velocity was calculated using a pulsed Doppler signal angled 45-60° to the vessel. The operator manually set the Doppler gate to record blood velocity from the proximal to distal wall of the artery. Integrated Doppler software was used to calculate the minimum and maximum velocities (Vmin and Vmax), time averaged maximum velocity (Tamax), and time averaged mean velocity (Tamean) for every heart beat. Blood velocity was recorded in real time using optical character reading software (Ammons Engineering) specially coded for operation through Labview (National Instruments). High-resolution B-mode imaging with a 5-10-MHz linear-array ultrasound transducer was used to measure changes in arterial diameter. All measurements were performed with the same position of the transducer and the arm. Magnification and focal zone settings were adjusted to optimize imaging of the proximal and distal vessel wall. Gain was kept constant throughout. Diameter measurements were taken at end-diastole. Brachial artery diameters were measured offline using semi-automated edge-detection software specially coded for use with Labview. Briefly, the operator selected a region of interest across the arterial wall. The true edges of the arterial walls were then represented by a line of best fit located by gradient-based detection with the region of interest. Arterial diameter was then estimated using a least-squared-error model fit.

Flow mediated dilatation

After 15minutes of rest in the seated position, images of the brachial artery diameter were recorded every 30 seconds for 5 minutes during baseline. Blood flow velocity measurements were recorded continuously. Results from all measurements recorded during baseline were averaged and are represented by the abbreviation *FMD base*. A pneumatic tourniquet (Hokinson) placed around the left forearm was then rapidly inflated (<1sec) to a pressure of approximately 100mmHg above systolic blood pressure. Brachial artery images were recorded every 30seconds during cuff-inflation for 5 minutes. Results from all measurements recorded during tourniquet inflation were averaged and are represented by the abbreviation *FMD cuff*. The pressure was then immediately released from the cuff to induce reactive hyperemia and brachial artery images were recorded every 3-5 seconds for 60 seconds. The peak diameter response is represented by the abbreviation *FMD peak*. Images were then collected every 30 seconds for an additional 4 minutes post tourniquet release. Results from all measurements recorded during the last 2 minutes of recovery were averaged to represent recovery values and are represented by the abbreviation *FMD recovery*. Heart rate (Biopac) and blood pressure (Datascope Accutor 3) were measured to account for systemic autonomic influences. A telethermometer (YSI) was used to record the temperatures of skin covering the following sites every 2minutes throughout the study: at the following sites: left brachial artery, right brachial artery, and left radial artery.

Graded exercise.

After recording baseline measures (*Exrs pre*) a handgrip ergometer was used to perform graded hand-grip exercise at two intensities: 1) 30% MVC, and 2) 50% MVC.

Exercise consisted of one constriction every 4 seconds for 4minutes. A handgrip ergometer was connected to a Biopac acquisition system using a force transducer interface. A three lead electromyograph (Biopac) was attached to the left brachial of the subject to determine the possible confounding influence of bicep muscle contraction. For the last 2 minutes of each exercise stage brachial artery images were collected every 10-20 second. Results from all measurements recorded during exercise were averaged and are represented by the abbreviations *Exrs 30 %MVC* and *Exrs 50 %MVC*. After the last stage of exercise a further 5 minutes of recordings were taken for all measurement. Results from all measurements recorded during the last 2 minutes of recovery were averaged and are expressed by the abbreviation *Exrs Recovery*.

Indirect local-heating.

After 10-15 minutes rest at room temperature in the seated position brachial artery diameter images were collected every 30 seconds for 5 minutes and the blood velocity was measured continuously. The subject's forearm was then immersed into ~40°C water for 5 minutes acclimatization prior to 5 minutes of data collection as above (Base 40). The above procedure was repeated with the forearm immersed in ~42°C. After 5 minutes acclimatization at 42°C, FMD was repeated. Baseline, ischemic, peak and recovery averaged measurement results are presented by the abbreviations: *42 Base, 42 Cuff, 42 Peak and 42 Recovery* respectively.

Statistical analysis

The blood velocity profile, brachial artery vasoactive (diameter) responses, blood pressure variables and heart rate were averaged for each protocol collection periods. FMD was expressed as the percent change in brachial artery diameter (%FMD). after

reactive hyperemia (FMD peak) relative to the average diameter of 5 minutes baseline (FMD base). Indirect heating plus FMD (%42°C FMD) was expressed as the percent change in brachial artery diameter following 42°C reactive hyperemia (42 FMD peak) relative to room temperature average diameter (FMD base).

To determine if gender differences influenced the results, female subjects from the reproducibility trial 1 and female subjects from the smoke control trial were pooled together, and the male subjects were similarly pooled together. Male baseline measurement variables (blood velocities, diameter, heart rate, blood pressures, skin temperatures) were compared against female baseline measurement variables using the two sample unequal variance 2-tailed T test. FMD dilation (%FMD & %42°C FMD) values were also compared to see if a gender interaction was evident using the two sample unequal variance 2-tailed T test.

Descriptive data for each stage of testing are expressed as mean (SD) for both the reproducibility group and smoke group. The reproducibility group brachial diameters, blood velocity variables, heart rates, blood pressure variables, and skin temperature values for trial 1 and trial 2 were compared using a 2-tailed paired T Test. Cigarette smoking was expected to have a negative effect on the vasoactive responses (brachial diameters) to increased blood velocity, but to have no effect on hyperemic (blood velocity) responses (Wever et al, 1998; Stadler et al, 1998). For this reason the brachial diameters for the smoking group control and smoking trials were compared using the 1-tailed paired T test, blood velocity variables, heart rates, blood pressure variables, and skin temperature values were compared using the 2-tailed paired T test The smoking group control trial diameters, blood velocity variables, heart rates, blood pressure

variables, and skin temperature values were compared to the reproducibility group trial 1 and trial 2 diameters using the 2-tailed two sample unequal variance T test. The smoking group smoke trial diameters were compared to the reproducibility group trial 1 and trial 2 diameters responses using the two sample unequal variance 1-tailed T test, blood velocity variables, heart rate, blood pressure variables, and skin temperature values were compared using the 2-tailed two sample unequal variance T test. The reproducibility group trial 1 and trial 2, and the smoking group control trial and smoke trial, vasoactive responses were correlated against the blood velocity parameters using linear regression analysis. The FMD values for the smoking group were compared using the paired T test. The FMD values for the smoking group control and smoke trials were compared to the reproducibility group trial 1 and trial 2 FMD values using the two sample unequal variance 1-tailed T test. The critical α value was set at 0.05.

Results

All subjects completed this study. Table 1 demonstrates the characteristics for the reproducibility group trial 1 and trial 2 and, for the smoking group for the control trial, and for the smoke trial pre- and post-smoking 1 cigarette. No significant differences for any baseline measure can be seen between groups or between trials. No significant differences were evident between males and females. There were also no significant differences between males and females for %FMD (12.4$\pm$4.5 vs 13.2$\pm$7.3 %FMD) or %42°C FMD (16.7$\pm$8.3 vs 27.0 $\pm$15.2 42°C %FMD) despite large mean differences for 42°C %FMD between the genders.

Table 2 shows the differences found in terms of percentages for brachial artery diameter, time averaged maximum blood velocity (Tamax), mean blood pressure, and

heart rate measurement variables between trial 1 and trial 2. There are no significant differences between trials for any diameter or Tamax measures. However, more variability in diameter measurements can be seen for exercise and heated conditions. Mean averaged blood pressure and heart rate were not significantly different between trials for the room temperature FMD test. More variability was seen during exercise and indirect local heating for the averaged blood pressure and heart rate recordings, but the changes were not significant. Not shown on table, for the exercise conditions there was also variability in force output between trials for each of the exercise intensities (0.04 ± 0.01 vs 0.06 ± 0.03 v for 30% MVC vs 50% MVC conditions, respectfully), but the differences were not significant. During 42°C indirect heating, the skin site of the imaged artery was significantly warmer when compared to room temperature baseline (30.2 ± 1°C at rest vs 36.8 ± 4.2°C during 42°C heating for trial 1; $P<0.01$). However, the skin site covering the imaged artery was not significantly hotter during the 42°C condition compared to the 40°C condition, and no significant differences were seen between trials.

Blood velocity increased in response handgrip exercise, local indirect heating and ischemia. Figure 2.1 shows no significant changes in the mean average Tamax responses to each stage of testing between trials. Figure 2.2 shows the vasoactive responses to changes in Tamax with each stage of testing. There are no significant differences between trials. Figure 2.3 illustrates the relationship between Tamax and diameter for the reproducibility group trial 1 and trial 2. This relationship is consistent over the two trials. Standard deviation bars can also be seen in this figure for room temperature base line diameter and Tamax measures and, for both room temperature and 42°C peak diameter responses. Despite the large group variances, a strong relationship is evident between

Tamax and diameter. Diameter measures have a stronger relationship with Tamax than any of the other velocity calculations, including: Tamaen, Vmin, or Vmax.

Figure 2.4 shows the room temperature FMD (%FMD) and 42°C %FMD. The 42°C %FMD test produced a significantly higher vasoactive response for both trial 1 (11.9±5.1 %FMD vs 18.4±10.4 42°C %FMD; P.<0.05) and trial2 (12.3±3.3 %FMD vs 18.2±4.4% 42°C %FMD; P.<0.05). There was little difference between the mean %FMD for trial 1 and the mean %FMD for trial 2 (3.7% difference), though the average individual absolute diameter response difference was higher (15.4%). %42°C FMD difference for the means between trial 1 and trial 2 was lower than for %FMD (-1.1%) though the average individual absolute difference was higher (37%) due to 1 subject demonstrating large variance between trials. %42°C FMD was also calculated using the 42°C average baseline diameter and the 42°C average ischemic (cuff inflated) diameter against the 42°C peak diameter, however using the room temperature average baseline diameter provided the most consistent response between trials.

Table 3 shows the differences found in terms of percentages for brachial artery diameter, Tamax, mean blood pressure, and heart rate measurement variables between control and smoke trials for the smoke group. Significant difference can be seen for the baseline diameter between control and smoke trials, and between pre-smoking and smoking for the smoke trial (P <0.05). This finding is better demonstrated in figure 2.6. Smoking 1 cigarette resulted in a significant constriction of the brachial artery when comparing pre-smoke to smoke during the smoke trial (0.33 vs 0.32 cm P<0.5), despite little variance in heart rate or blood pressure variables. Greater variance can be seen with Tamax when compared to the reproducibility study group. Little variance can be seen

with the diameter responses to both exercise intensities. There was also little variance seen during the heated conditions, though the peak 42°C diameter response was slightly lower (0.40±0.4 vs 0.39±0.3 cm) but not significant. There was little variance in mean blood pressure or heart for the different conditions.

Figure 2.5 shows low variability in the mean average Tamax responses to each stage of testing between control and smoke trials. Figure 2.6 shows little effect on the mean vasoactive responses to each stage of testing apart from during the room temperature FMD. Comparison of the smoke trial to the control trial shows that smoking one cigarette resulted in a significant reduction in the peak vasoactive response to room temperature hyperemia (0.37±0.4 v 0.34±0.4 cm $P < 0.05$). The peak 42°C diameter response was reduced by only 2.4%, not a significant reduction.

Figure 2.7 shows the relationship between mean average Tamax and mean diameter for the smoke group control and smoke trials. Despite the large group variances, a strong relationship is demonstrated between Tamax and diameter for the control trial ($R_2 = 0.87$). Smoking decreased the strength of this relationship ($R_2 = 0.72$) due to a decreased peak FMD diameter, but the relationship was consistent between trials. Figure 2.8 compares room temperature FMD to 42°C FMD. Room temperature FMD was significantly attenuated by 42.0% (11.6±6.7 v 6.7±3.8 %FMD $P<0.5$). 42°C FMD was no attenuated (22.2±5.4 v 24.3 ±12.7 %FMD).

Discussion

Reproducibility Study

This study found comparable flow mediated dilation (FMD) reproducibility between trials as observed by Corretti et al (2002) %FMD values for the reproducibility

group were comparable when trial 1 (11.9±5.1 %FMD) was compared to trial 2 (12.3±3.3 %FMD; 3.7% group mean difference). Numerous factors can contribute to the remaining %FMD variability, including equipment related, operator related, and physiological influences. To confront equipment related and operator related influences the protocol administered for each protocol was standardized and all analyses were performed offline. Physiological influences were minimized by performing each trial under fasting or low-diet conditions and at the same time of day and within 48 hr of each other. Subjects were also asked not to consume any caffeine, medications or drugs with known vascular effects. The testing environment was also kept at standardized room temperature and noise free. The fluctuations seen in the FMD response may have thus been due to daily physiological fluctuations.

A novel finding pertained to the 42°C FMD test producing a significantly greater FMD effect size (18.4 ±10.4 %FMD trial 1; 18.2±4.4 %FMD trial 2) than FMD ($P<0.05$), and being more reproducible (-1.1% group mean difference) than %FMD, at least when the group mean %42C FMD values were compared. This way of reporting reproducibility may be somewhat misleading however since the average individual absolute diameter difference was actually higher for the 42°C FMD test when compared to FMD (15.4% v 37.1% average individual absolute diameter difference) due to high variability for 1 subject. Thus, whilst %42°C FMD may give a greater effect size, the test may not be more sensitive than FMD.

An additional finding was a strong and consistent relationship between time averaged maximum (Tamax) blood velocity and the brachial artery vasoactive response ($R_2 = .92$, $P.<0.5$ for trial 1; and $R_2 = .88$, $P.<0.5$ for trial 2, no significant difference

between trials). The brachial artery diameter demonstrated a stronger relationship with Tamax than any other blood velocity stimuli calculated (i.e. Vmin, Vmax or Tamean). This study took a novel approach at investigating the relationship between blood velocity and the vasoactive response. Blood velocity was manipulated using progressive intensity handgrip exercise, indirect local-heating, and ischemia. The exercise mode and protocol chosen was such that increased sympathetic activity was unlikely to occur and become a confounding factor. Similarly, the local indirect-heating protocol used was based on preliminary pilot work in our laboratory showing the protocol not to have a direct heating effect over the skin site of the imaged artery. This study found that the two stimuli chosen were able to manipulate blood velocity over a controlled period of time, and in turn use these stimuli to control vascular tone.

Wall shear stress is thought to be one of the most important local factors governing vascular tone (Koller & Kley, 1990). The primary mechanism thought responsible for the vasoactive response to reactive hyperemia at room temperature is the release of NO from the vascular endothelium in response to increased sheer stress (Koller & Kaley, 1990; Koller & Sun, 1994; Moncada & Higgs, 1993). This NO gas is continuously released from the endothelium where it is synthesized from the precursor L-arginine in a reaction catalyzed by nitric oxide synthase (eNOS isoform in endothelium) (Baron *et al*, 1999). The mechanism is thought to be entirely local and due to stimuli initiated from the blood velocity profile, i.e. shear stress.

Although the data are not shown for all other blood velocity parameters (i.e. Tamean, Vmin, Vmax), the findings from this study suggest Tamax to be the primary blood velocity stimuli responsible for triggering shear stress across the artery wall. No

relationship was seen between the diameter and the minimum velocity. However, it must also be recognized that blood velocity is not uniform across the artery (i.e not plug flow) but is parabolic. The Doppler gate for this study was placed across the entire vessel and hence averaged Tamax across the entire vessel. Because we did not have blood velocity measures at the vessel wall we did not calculate actual shear stress. Further study is need to determine what the relative contribution of the blood velocity parameters to shear stress is as the profile moves from the center of the vessel to the wall of vessel. This study also did not look at the relationship between shear stress and time, i.e. does a given level of shear stress promote a greater level of vasodilatation if the shear stress is maintained over time?

Smoking Study

From the over 4000 substances produced by incomplete combustion of cigarettes, it is not known which are the most potently involved in the intoxication of endothelial function, though carbon monoxide and nicotine are the most likely suspects (Patel & Kent, 1998). Long-term cigarette smoking can promote intimal hyperplasia and produce morphological changes within the endothelium (Douglas et al, 1994). Short-term the oxidants from cigarette smoke promote excessive production of superoxide anions and the oxidation of low-density lipoproteins. In particular, this excessive production of superoxide anions is thought to result in the degradation of NO before its interaction with target cells (Wever et al, 1998). The ramifications of cigarette smoking, as regards the ability of the vascular endothelium to regulate tone, have been demonstrated in a number of studies (Murohara et al, 1994; 1997; Pittilo, 2000). For this reason smoking was

chosen in this study as the most suitable non-invasive inhibitor of vascular NO production.

After smoking 1 cigarette the smoking group had significantly reduced baseline diameters when compared to their pre-smoking diameters during the smoke trial (.33±0.03 vs .32±.04 cm P<0.5). Smoking 1 cigarette also markedly attenuated %FMD (11.6±6.7% vs 6.7±3.8 %FMD, P<0.5; 42.1% difference). However, smoking did not attenuate %42°C FMD and had no effect on the Tamax-diameter relationship (see figure 7). These points will be discussed in the following text.

Progressive intensity exercise. Figure 1 shows a marked increase in Tamax blood velocity with increasing exercise intensity. In Figure 3 this relationship can be seen to be consistent across conditions. Smoking one cigarette did not alter the Tamax blood velocity response to handgrip exercise (see Figure 5) and the relationship between Tamax and brachial artery vasoactivity was not altered (see figure 7).

Skeletal blood flow during exercise is thought to be regulated by a number of mechanisms, with the relative importance of each dependent on the mode and intensity of exercise. These mechanisms can be separated into three major regulatory systems: (I) systematic control, encompassing both neural and humoral regulation, (II) the muscle pump, and (III) local control by myogenic or vasoactive factors.

NO and adenosine are credible candidates for local regulation of skeletal muscle blood flow (Radegran & Hellsten, 2000), with both substances being shown to increase in skeletal muscle cells and interstitial fluid during exercise. The exact role and capacity of NO is yet to be clearly determined, and it remains unclear whether the exercise-induced NO formation in muscle originates from endothelial NOS (eNOS) in the microvascular

endothelium, or from neuronal NOS (nNOS) in nerve and muscle fibers (Kobzik et al, 1994; Radergran & Hellsten, 2000). The later pathway may be responsible for the tight regulation of nutritive flow to metabolically active sites as described recently by Clark et al (2001). This nNOS pathway is still understudied however. What is more, whilst it is a real possibility that nNOS may be the precursor to arteriole NO release during exercise, the effect of this synthase on conduit artery tone is undetermined, and it is possible that this pathway is more involved with the vascular bed directly perfusing the metabolically active skeletal muscle than with conduit arterial blood flow.

The possibility exists that central mediating factors influenced the results. However, the muscle group exercised was small and unlikely to have provoked systemic changes, and whilst there was more variability in heart rate and blood pressure during exercise compared to baseline the changes were not significant

The dilation of larger conduit arteries upstream from the area of demand is assumed to represent an important vascular response for the increased demand in blood flow to the microvessels supplying the active muscle(s) (Lash, 1994). Since the dilation of microvessels raises blood velocity through upstream conduit arteries, it is possible that FMD represents the mechanism in effect for these upstream arteries. The fact that smoking 1 cigarette did not alter the Tamax-diameter relationship may not be inconsistent with the last statement.

Thomas (2002) speculated that sympathetic control may compensate in the absence of effective NO regulation of vascular tone, suggesting that a delicate balance exists between NO and sympathetic regulation of vascular tone. Put into the context of the current study, it is possible that the prolonged blood velocity stimuli produced

through exercise may have allowed time for adaptive changes through this integrative system. Indeed, upregulation of one system in response to a deficiency of another has also been demonstrated by a number of other investigators over recent years (Beverelli et al 1997; Doni et al 1988; Nishikawa et al 2000). Smoking one cigarette may have had the marked effect on room temperature FMD because the stimuli produced a bolus effect in terms of the blood velocity profile. The time window was such that compensatory mechanism(s) may have been unable to act. Since the time course for peak vasoactive responses with each condition were not measured for this study, further speculation is not possible.

Indirect local-heating. A strong relationship was observed between the vasoactive responses to changes in Tamax induced through the progressive indirect local heating (see Figure 3). This relationship was consistent with those found using progressive exercise and ischemia to induce changes in blood velocity. However, similar to the findings from the exercise protocol, smoking 1 cigarette had no influence on this relationship.

The mechanism(s) responsible for vasodilatation with local heating are not fully understood, but endothelial NO production is thought to play a central role. NOS inhibition through NG-nitro-L-arginine methyl ester (L-NAME) administration attenuates cutaneous vasodilation induced via local skin heating (Kellogg et al, 1999). Recent evidence suggests that NO serves a permissive role to some unknown neurotransmitter in creating a neurogenic reflex to increase blood flow to the heated area (Farrell & Bishop, 1995). There may also have been decreased affinity of alpha2-receptors for norepinephrine (Pergola et al, 1993) - though this particular mechanism is thought to

account for only 10% of the vasoactive response (Pergole et al, 1993). To complicate the issue, the role of local heating on conduit artery tone is still not established. Nonetheless, these aforementioned studies have looked at *direct* local heating; the current study used local heating to *indirectly* increase *blood flow demand* down-stream so that *blood velocity* upstream is *indirectly* increased.

It was hypothesized that blocking the vascular eNOS dependant NO pathway would thus attenuate the vasoactive response expected with increased blood velocity. However, smoking one cigarette did not attenuate the response seen. This lack of attenuation may be due to fact that the skin site of the imaged artery did actually demonstrate a significant heating effect during the smoking trial (28.8$\pm$.6 vs 33.1$\pm$1.1°C for room temperature baseline and 42°C baseline conditions respectively; P>0.01). However, a greater heating effect was seen at the skin site of the imaged artery during the control trial than for the smoke group, and thus it is unlikely that the results can be entirely explained by this confounding factor.

Assuming that smoking one cigarette blocked eNOS dependant NO production, eNOS derived NO either does not play a prominent role in conduit artery vasodilatation, or other compensatory mechanisms may have had an effect. However, no significant changes in heart rate on blood pressure were seen with any of the indirect local-heating conditions.

%FMD vs %42C FMD. The %FMD for the smoking group (11.6$\pm$6.7% FMD) before smoking was similar to that found for the control group and above the cut-off point recommended by the CANDEV study (Accini et al, 2001). However, whilst smoking one

cigarette markedly attenuated %FMD (42.1% reduction), %42°C FMD was not attenuated.

NO may play a primary role in mediating vascular tone, however the subjects chosen may have been young and healthy enough for compensatory mechanisms (due to prolonged reactive hyperemia compared to %FMD) to promote an apparently normal vasoactive response.

The current results may also simply imply that one cigarette was not a strong enough dose to completely prevent NO mediated FMD. Indeed, the attenuated room temperature FMD response after smoking one cigarette was less than that previously reported. Lekakis et al (1998) found a FMD response of 11. $\pm$3.5% in 10 healthy 36 $\pm$7 year old subjects before smoking one cigarette, FMD was recorded at 3.7%$\pm$4.9% immediately after smoking one cigarette. Alternatively, an equally simple explanation for the lack of FMD response with 42°C heating compared to room temperature FMD pertains to the fact that the 42°C FMD test was the last conducted in this series of experiments. According to Lekakis and colleagues (1998) cigarette smoking markedly attenuates the FMD for up to 60 minutes following the smoking of one cigarette, followed by the sharpest part of the recovery slope at 60-90 minutes. This current study was unable to randomize the test conditions based on preliminary data, and this aforementioned finding may largely explain the lack of response see due to the 42°C measurement being taken during the 60-90min post-smoking time frame.

Summary and Conclusions.

Blood velocity was manipulated with ischemia, progressive intensity handgrip exercise and local indirect-heating. A strong and reproducible Tamax-diameter

relationship was found. An additional and novel finding of this study was a greater effect size and higher reproducibility for the FMD test when coupled with 42°C indirect local heating. However, whilst smoking one cigarette had a marked effect on %FMD response there was no attenuation recorded for 42°C %FMD. In addition, smoking 1 cigarette had no effect upon the vasoactive response to either progressive intensity handgrip exercise, or to indirect local -heating. These findings may be attributed to: 1) the vasoactive response to exercise and local-heating may not be attributable to vascular endothelial release of NO through the eNOS pathway; 2) exercise and indirect local-heating may have resulted in either central mediating effects or, provoked a diameter response through a different NO-regulated mechanism; 3) the prolonged exercise and local indirect-heating stimuli may have transiently provoked adaptive integrative compensatory mechanisms.

In conclusion, handgrip exercise and local indirect heating have potential to serve as tools for screening vascular health, however the use of these potential tools may be supportive to the standard FMD test. The 42°C FMD test has the potential to improve the sensitivity and reliability of the FMD test, though the limitations of this study first need to be addressed. Future studies would do well to initially focus on: 1) repeating the 42°C FMD test immediately after cigarette smoking; 2) measure the time course to peak vasoactive response to reactive hyperemia before and after cigarette smoking.

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Table 1. Characteristics for both groups of subjects: age, and resting heart rate and blood Values for the different sessions.

	Reproducibility Group		**Smoke Group**		
	Trial 1	**Trial 2**	**Control**	**Smoke**	
				Pre-Smoke	Smoke
N	6 (3male/3female)		6 (3male/3female)		
Age (yrs)	22.7+2.9		20.2+-.8		
Heart Rate (bpm)	68.3(12.1)	68.1 (12.8)	75.8(12.3)	76.75(18.0)	78.4(13.1)
Systolic BP (mmHg)	109.7(15.5)	114.4(6.6)	119.1(11.5)	114.4(6.6)	115.3(5.1)
Diastolic BP (mmHg)	63.6(6.4)	64.8(5.7)	68.25(5.5)	72(1.1)	69.(6.4)
Mean BP (mmHg)	83.0(10.1)	84.2(8.5)	86.3(4.6)	88.6(7.3)	86.1(5.2)

Values are mean(SD)

Table 2. Mean differences (%) for measurement variables between Trial 1 and Trial 2

Conditions	Diameters (cm)	Tamax (cm/s)	Mean BP (mmHg)	HR (bpm)
Base	0.29	17.71	1.23	-0.30
FMD Cuff	-0.60	61.02	-0.16	2.14
FMD Peak	0.68			
FMD Post	2.18	26.82	5.23	-2.72
Exrs Pre	4.98	12.25	2.97	-3.73
Exrs 30%	3.65	5.78	9.13	-2.05
Exrs 50%	1.35	12.48	5.95	-2.29
Exrs Post	-0.74	9.65	5.83	0.58
Base 40	2.76	2.67	1.27	-1.13
42 Base	4.05	3.30	1.92	-0.25
42 Cuff	2.00	7.41	6.50	-2.20
42 Peak	0.12			
42 Recovery	-0.28	-0.22	-0.02	1518

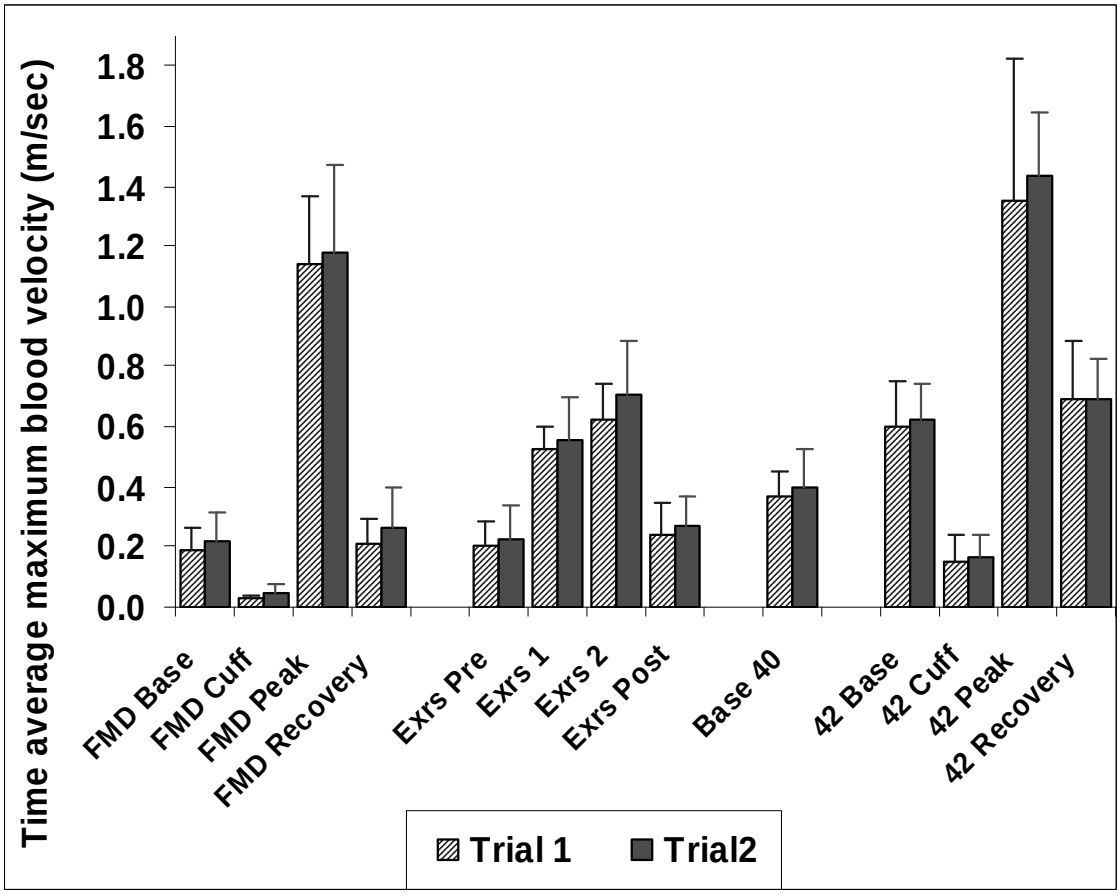
Note: peak values represent the peak responses to reactive hyperemia and are not mean values. Note: no peak control values for FMD peak and 42C Peak; Tamax, Mean BP and Mean HR readings were not sensitive enough to record single peak values. FMD = flow mediated dilation. Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Table 3. Mean differences (%) for measurement variables between control and smoke trials.

Conditions	Diameter (cm)	Tamax (cm/s)	Mean BP (mmHg)	HR (bpm)
Pre	0.05			
Base	-4.04	-23.73	-0.32	3.42
FMD Cuff	-1.58	-38.33	0.66	-0.33
FMD Peak	-8.22*			
FMD Post	-6.78*	-37.20	-4.72	0.28
Exrs Pre	-1.87	-38.10	4.07	1.82
Exrs 30%	0.86	11.62	1.28	1.64
Exrs 50%	-0.22	-1.70	-4.44	-1.38
Exrs Post	-3.86	-21.18	1.65	-6.70
Base 40	1.63	-5.85	0.76	0.34
42 Base	0.37	1.07	-1.42	2.72
42 Cuff	0.82	56.23	-1.55	3.60
42 Peak	-2.39			
42 Recovery	-2.10	12.48	-3.90	1.67

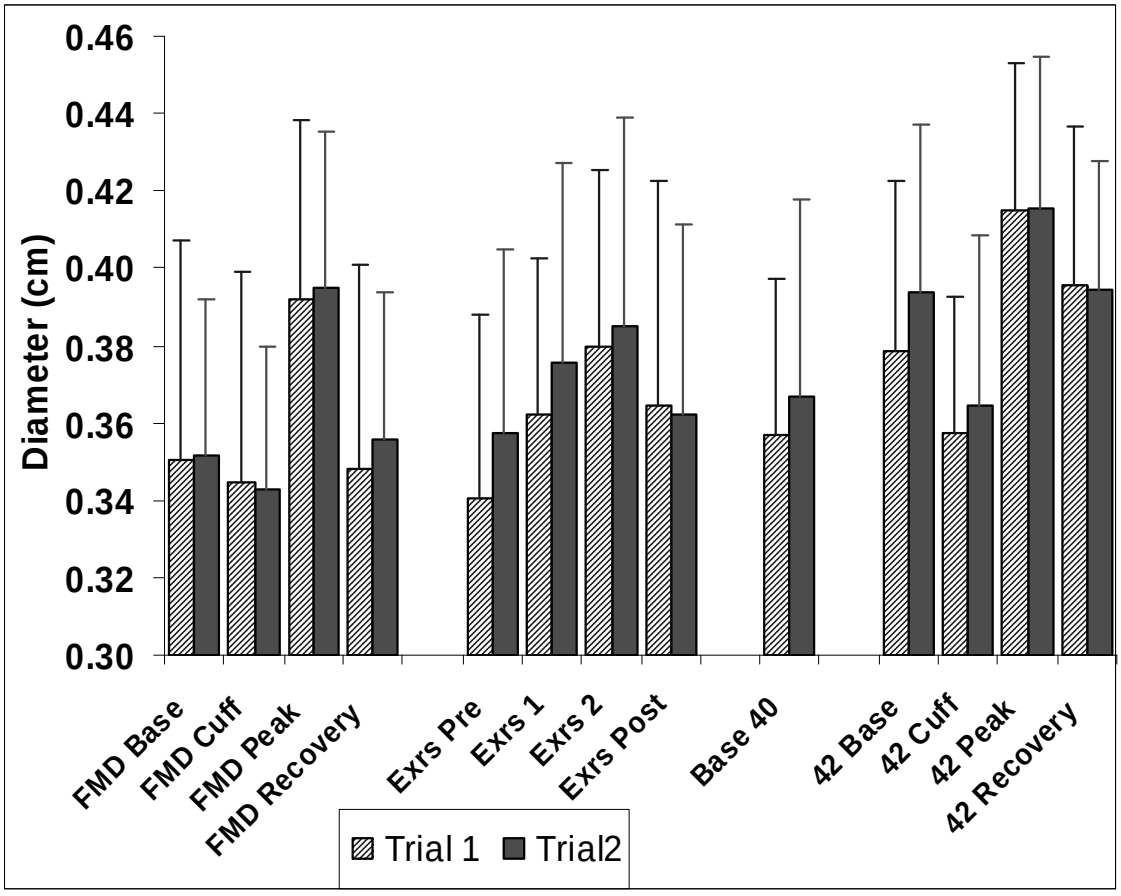
* = significant difference from the paired T Test (P <.02). Note: Peak values represent the peak responses to reactive hyperemia and are not mean values. Note: no peak control values for FMD peak and 42C Peak; Tamax, Mean BP and Mean HR readings were not sensitive enough to record single peak values. FMD = flow mediated dilation. Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Figure 2.1. Comparison of time average maximum velocities between trials for each stage of testing. Values are means (SD).



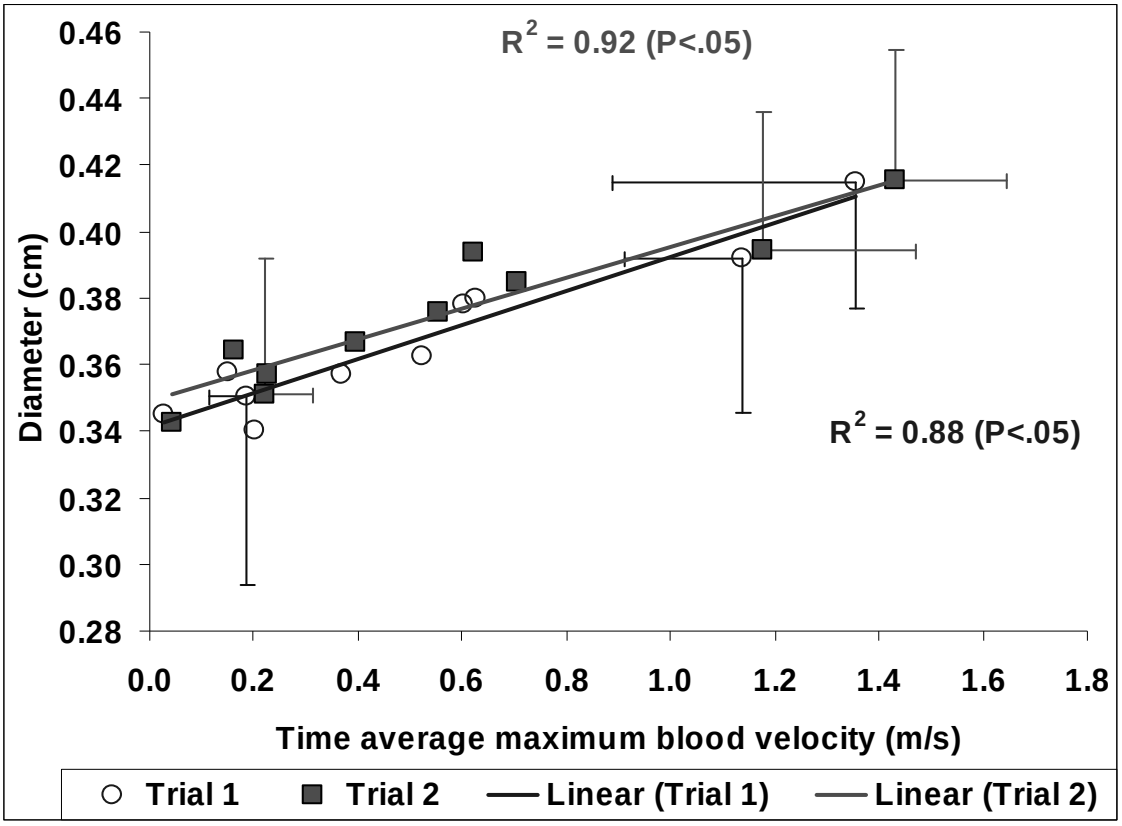
Values are reproducibility group means (SD). FMD = flow mediated dilation. Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Figure 2.2. Comparison of mean diameters between trials for each stage of testing. Values are means (SD).



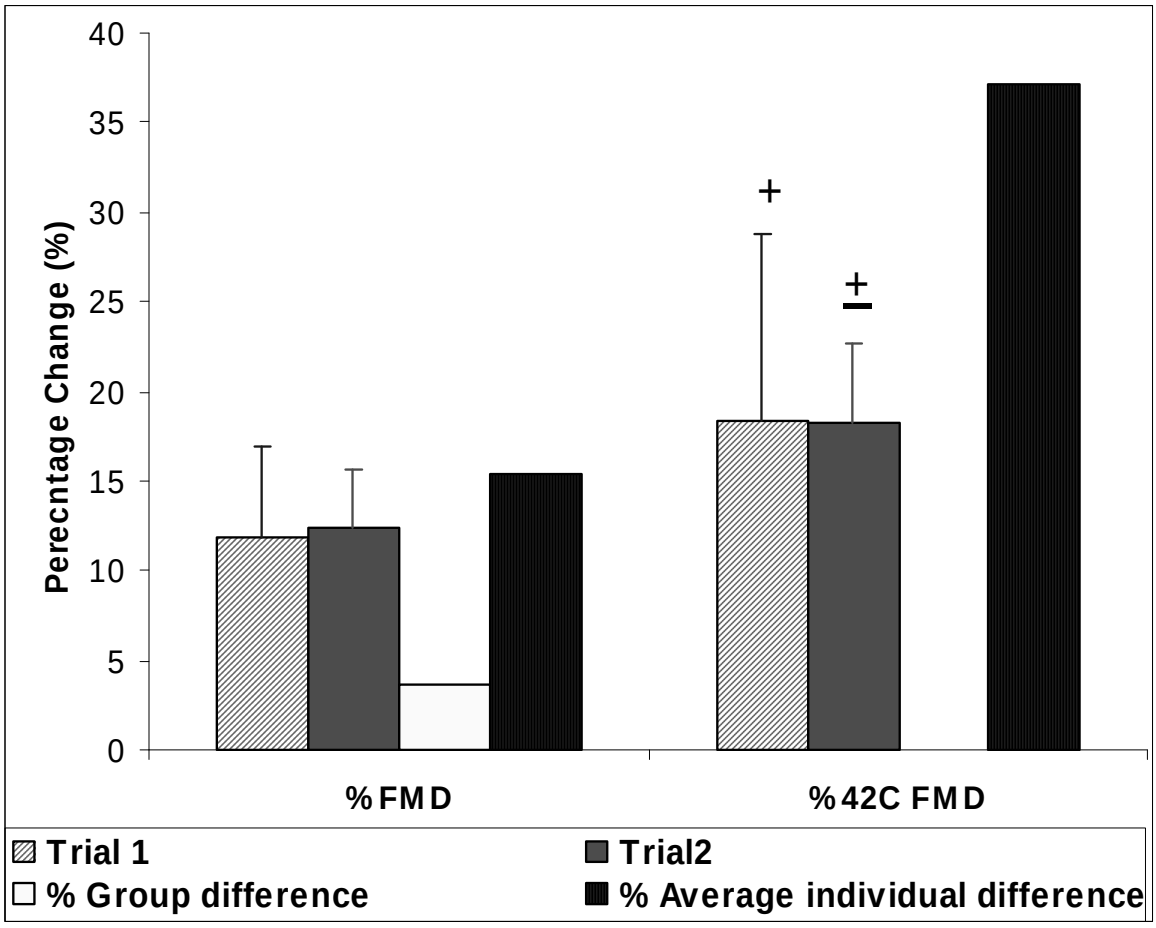
Values are reproducibility group means (SD). FMD = flow mediated dilation. Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Figure 2.3. Time averaged maximum velocity plotted against diameters for Trial 1 and Trial 2.



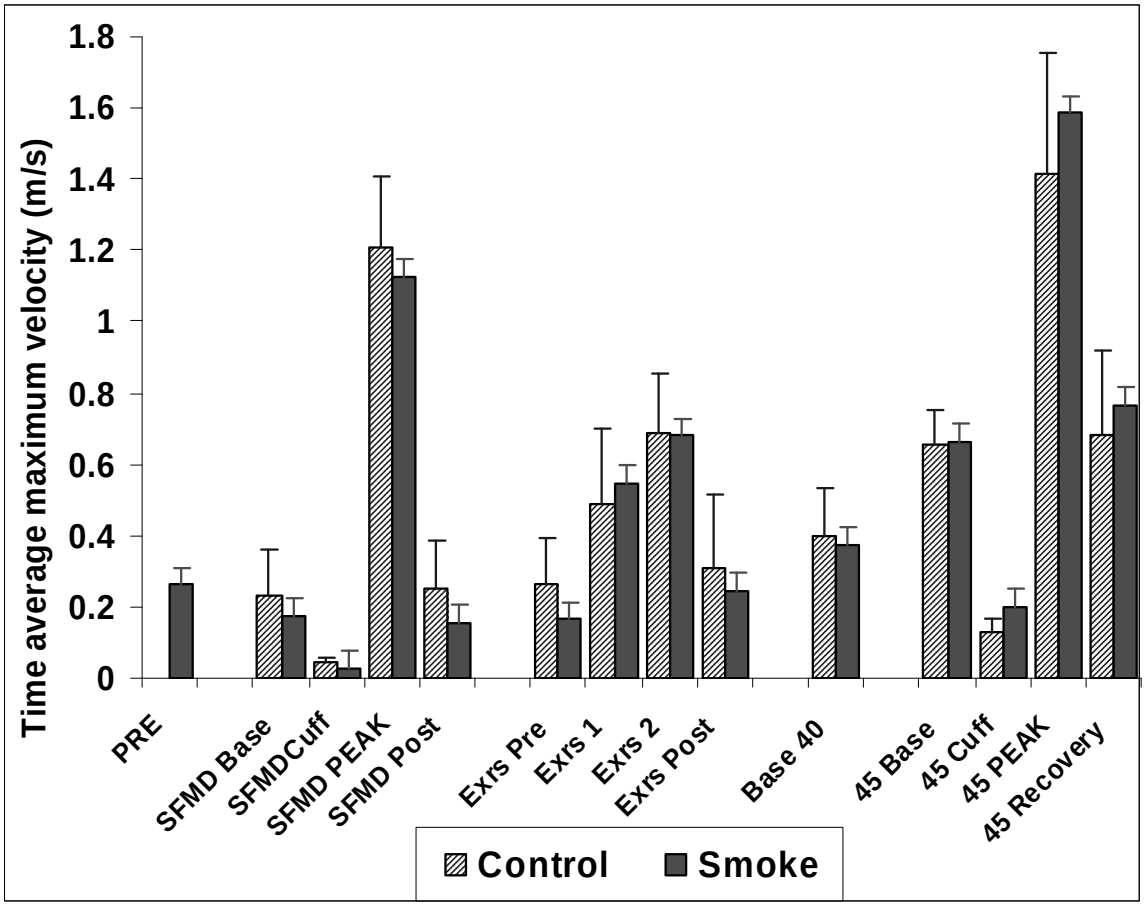
Values are reproducibility group means. Each data point represents the group mean blood velocity and corresponding diameter response to each of the study conditions as shown in figures 1 and 2. Representative standard deviation bars represent the group standard deviations for room temperature and 42°C peak responses.

Figure 2.4. Comparison of room temperature flow mediated dilation against 42°C flow mediated dilation for trial 1 and trial 2.



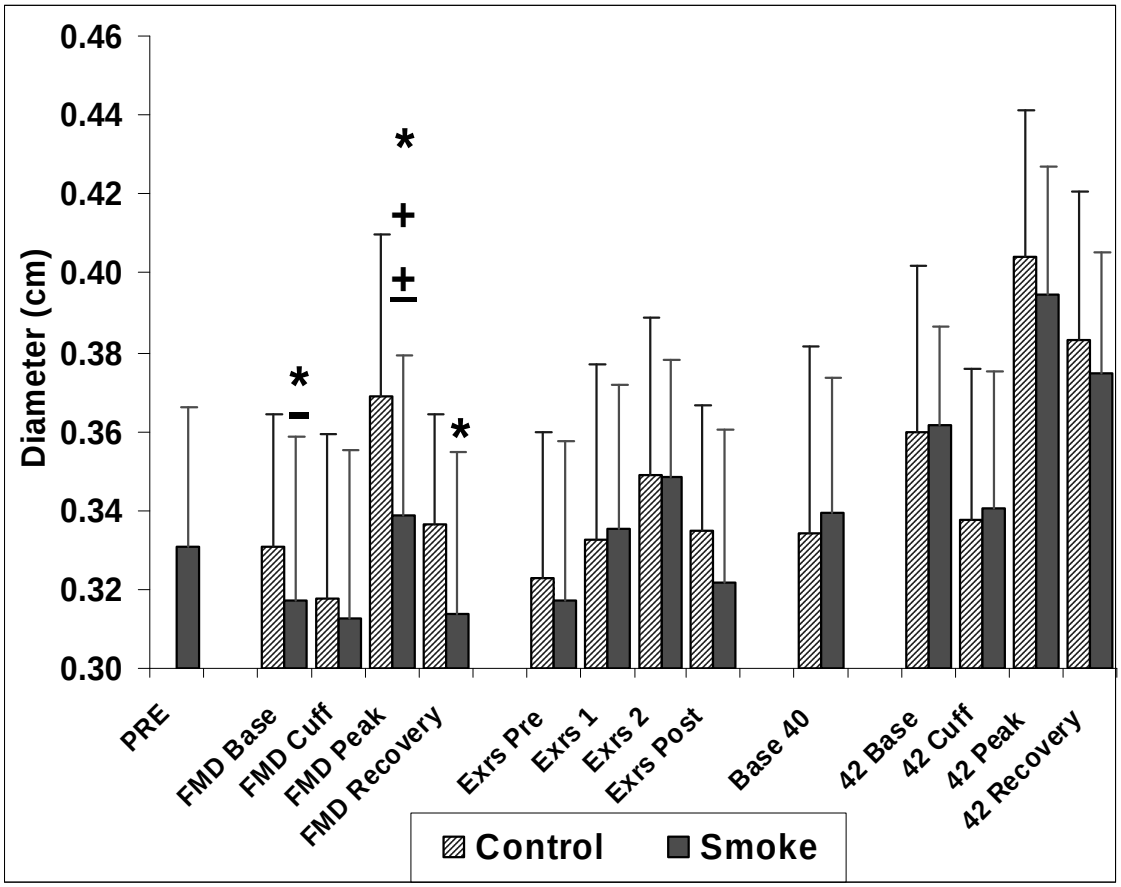
%FMD = percentage difference between room temperature average diameter and room temperature average peak diameter response. %42C FMD = percentage difference between room temperature average diameter and 42°C peak average diameter. % Group difference = difference between the group means between trials. % Average individual difference = the average absolute diameter individual difference + = Significant difference from Trial 1 %FMD by the paired T-test ($P<0.05$). $\pm$ = Significant difference from Trial 2 %FMD by the paired T-test ($P<0.05$).

Figure 2.5. Comparison of time average maximum blood velocities for control and cigarette smoking trials.



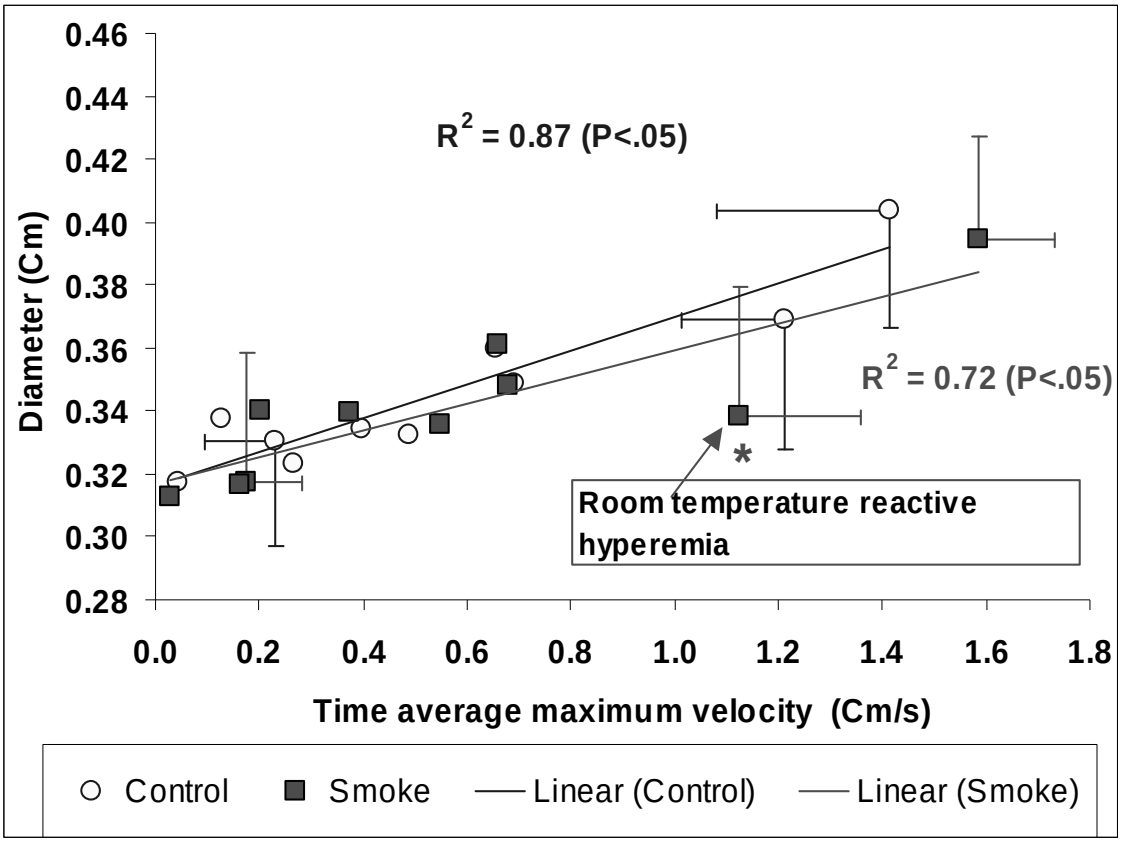
Values are smoke group means (SD). Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Figure 2.6. Comparison of diameters for control and cigarette smoking trials.



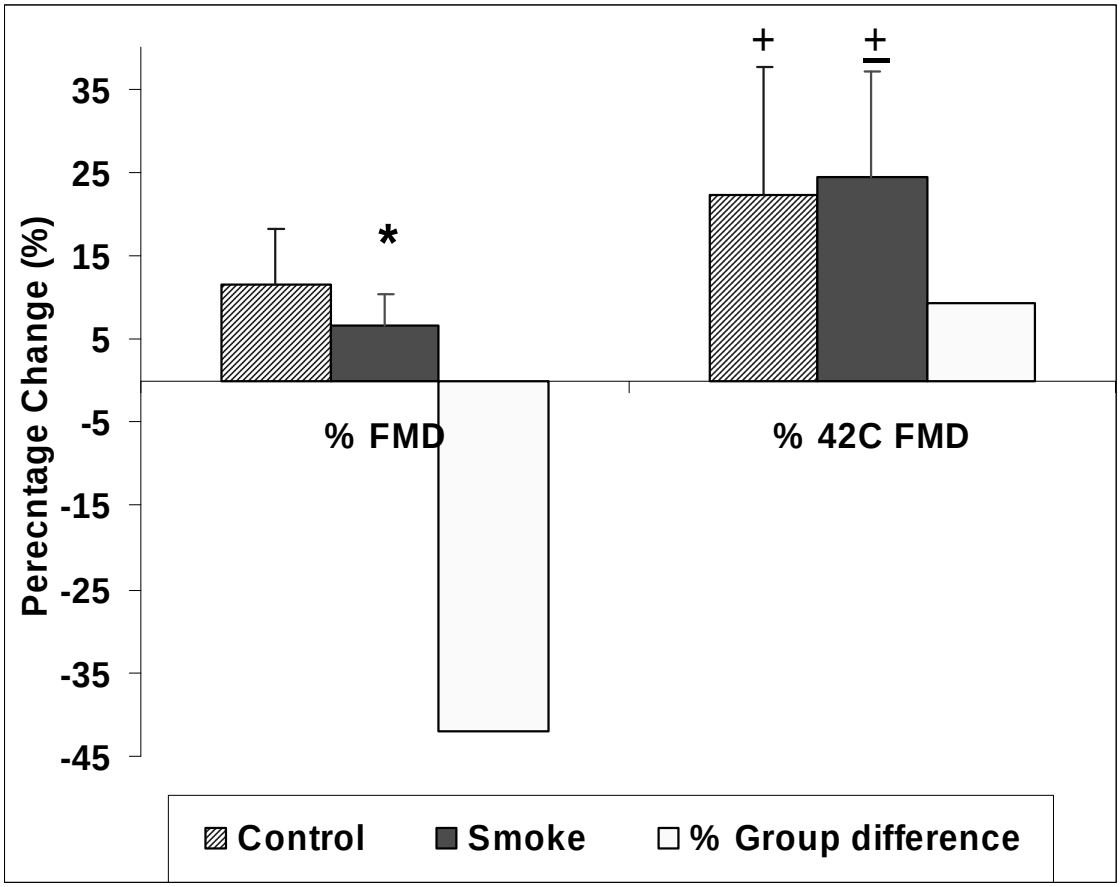
Values are smoke group means (SD).* = significant difference between smoking and control trial by the paired T test (P <.05). * = significant difference from smoke trial pre diameter by the paired T test (P <.05). + = significant difference from reproducibility group trial 1 by the independent T test (P <.02). ± = significant difference from reproducibility group trial 2 by the independent T test (P <.03). Exrs = exercise. Base 40 = 40°C indirect local heating. 42°C = 42°C indirect local heating.

Figure 2.7. Time average maximum blood velocities v diameters for control and cigarette smoking trials.



Values are means (SD). * = significant diameter difference from the paired T test (P <0.05). Values are smoking group means. Each data point represents the group mean blood velocity and corresponding diameter response to each of the study conditions as shown in figures 5 and 6. Representative standard deviation bars represent the group standard deviations for room temperature and 42°C peak responses.

Figure 2.8. Comparison of room temperature flow mediated dilation against 42°C flow mediated dilation: Effects of cigarette smoking



%FMD = percentage difference between room temperature average diameter and room temperature average peak diameter response. %42°C FMD = percentage difference between room temperature average diameter and 42°C peak average diameter. % Group difference = difference between the group means between trials. % Average individual difference = the average absolute diameter individual difference + = Significant difference from control %FMD by the paired T-test (P0.05). ± = Significant difference from smoke %FMD by the paired T-test (P0<.05).

CHAPTER 3

CONCLUSIONS

Blood velocity was manipulated with ischemia, progressive intensity handgrip exercise and local indirect-heating. A strong and reproducible blood velocity (Tamax)-diameter relationship was found. An additional and novel finding of this study was a greater effect size and higher reproducibility for the flow mediated dilation (FMD) test when coupled with 42°C indirect local-heating. However, whilst smoking one cigarette had a marked effect on room temperature FMD there was no attenuation recorded for 42°C FMD. In addition, smoking 1 cigarette had no effect upon the vasoactive response to either progressive intensity handgrip exercise, or to local indirect-heating. These findings may be attributed to the following explanations: 1) the vasoactive response to exercise and indirect local-heating may not be attributable to vascular endothelial release of NO through the eNOS pathway; 2) exercise and local indirect-heating may have resulted in either central mediating effects or, provoked a diameter response through a different NO-regulated mechanism; 3) the prolonged exercise and local indirect-heating stimuli may have transiently provoked adaptive integrative compensatory mechanisms.

Since no significant systemic responses were recorded, and the blood velocity stimuli (ischemia, handgrip exercise, & local indirect-heating) chosen ‘indirectly’ increased blood velocity through the brachial artery, explanation 3) was chosen as the most plausible.

The primary mechanism thought responsible for the vasoactive response seen after ischemia (i.e. FMD) at room temperature is the release of NO from the vascular endothelium in response to increased sheer stress (Celermajer et al 1992). The mechanism is thought to be entirely local and due to stimuli initiated from the blood velocity profile, i.e. sheer stress. This study used ischemia, progressive intensity handgrip exercise and local indirect-heating to manipulate blood velocity without evoking systemic effects. A strong relationship was found between blood velocity (Tamax) and brachial artery diameter. However, despite significantly attenuating room temperature FMD cigarette smoking did not significantly influence this relationship. It was concluded that the prolonged reactive hyperamia in response to handgrip exercise and indirect local-heating may evoke compensatory mechanisms, at least in the occasionally smoking but young healthy subjects used for this study. Indeed, upregulation of one system in response to a deficiency of another has been demonstrated by a number of investigators over recent years (Beverelli et al 1997; Doni et al 1988; Nishikawa et al 2000). Smoking one cigarette may have had the marked effect on room temperature FMD because the stimuli produced a bolus effect in terms of the blood velocity profile. The time window was such that compensatory mechanism(s) may have been unable to act.

The current results may also simply imply that one cigarette was not a strong enough dose to completely prevent NO mediated FMD. Indeed, the attenuated room temperature FMD response after smoking one cigarette was less than that previously reported (Lekakis et al 1998). Alternatively, the handgrip exercise and indirect local-heating protocols were conducted after the room temperature FMD test, and the effects of cigarette smoking may have worn off.

In conclusion, handgrip exercise and local indirect heating have potential to serve as tools for screening vascular health, however the use of these potential tools may be supportive to the standard FMD test. The 42°C FMD test has the potential to improve the sensitivity and reliability of the FMD test, though the limitations of this study first need to be addressed. Future studies would do well to initially focus on: 1) repeating the 42°C FMD test immediately after cigarette smoking; 2) measure the time course to peak vasoactive response to reactive hyperemia before and after cigarette smoking.

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