IN VIVO BLOOD VELOCITY PARAMETERS THAT CONTRIBUTE TO FLOW-MEDIATED DILATION

by

LEE STONER

(Under the Direction of Kevin McCully)

ABSTRACT

Flow-mediated dilation (FMD) is the auto-regulation of blood vessel size in response to flow-induced increases in shear stress. FMD is governed by the vascular endothelium, an important monolayer which also regulates vascular homeostasis. Tests of FMD offer the potential to predict future cardiovascular disease event. The usefulness of these tests can be improved if FMD is normalized to the shear stimulus.

The purpose of study one was to determine the importance of velocity acceleration to FMD. FMD was measured prior to and following induced increases in velocity acceleration. Mean blood velocity was kept constant between conditions. Fourteen physically active, young (26±5 years) male subjects were tested. Blood flow to the forearm was manipulated using progressive local heating and handgrip exercise. Brachial artery blood velocities and diameters were measured using ultrasound. Velocity acceleration was increased by inflating a tourniquet around the forearm to 40 mmHg. Hierarchical linear modeling (HLM) was used to estimate change in diameter with repeated measures of shear rate nested within each subject. The shear rate-diameter slope was used to represent FMD. When velocity acceleration was increased FMD was attenuated by 11% (P = 0.015).
The second study assessed whether peak- and time integrated-shear rates independently predict FMD. Eleven physically active, young (2±5 years) male subjects were tested. Each subject was tested under transient and steady-state shear rate conditions. During the transient condition, shear rate was increased using four down-stream ischemic durations (2, 4, 6 & 10 min). During the steady-state condition, shear rate was manipulated using progressive local heating and handgrip exercise. HLM was used to estimate change in diameter with repeated measures of shear rate nested within each subject. When accounting for both time integrated and peak shear rates, FMD did not significantly differ between transient and steady-state conditions (P = 0.067). Collectively, these findings suggest that the velocity profile and the peak shear response contribute to the shear stimulus for FMD.

INDEX WORDS: Flow mediated dilatation, velocity acceleration, blood velocity, blood flow, shear stress, shear rate, flow pulsatility, pulsatility index, flow turbulence, ischemia, forearm exercise, forearm heating.
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DEDICATION

I dedicate this work to my grandparents, David and Kathleen Stoner. Without their help and support I would not be arriving at this moment in my life.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>vi</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**
   - Study Significance: 3
   - Purpose Statement: 4
   - Hypotheses: 4

2. **LITERATURE REVIEW**
   - Anatomy and Functions of the Vascular Endothelium: 5
   - Stimuli Regulating Endothelial Function: 10
   - Shear Stress Estimation: 13
   - Shear Stress Mediated Control of Endothelial Function: 18
   - Flow-Mediated Dilation: 22
   - Influence of the Blood Flow Profile on Endothelial Responses: 30
   - FMD with Transient vs. Steady-State Shear Stress: 41
   - Importance of the Blood Flow Profile to Health: 46
   - Assessments of Endothelial Function: 49
3 THE IMPORTANCE OF VELOCITY ACCELERATION TO FLOW-MEDIATED DILATION ..................................................................................................................65
  Abstract ...................................................................................................................65
  Introduction .............................................................................................................67
  Methods ...................................................................................................................68
  Results .....................................................................................................................76
  Discussion ...............................................................................................................79
  References ..............................................................................................................84
  Figure Legends ........................................................................................................91
  Tables ......................................................................................................................93
  Figures .....................................................................................................................95

4 EVALUATION OF THE DURATION OF SHEAR STIMULUS ON FLOW-MEDIATED DILATION ......................................................................................101
  Abstract .................................................................................................................102
  Introduction ...........................................................................................................103
  Methods ...................................................................................................................104
  Results ...................................................................................................................112
  Discussion .............................................................................................................115
  References .............................................................................................................119
  Figure Legends ......................................................................................................126
  Tables ....................................................................................................................128
  Figures ...................................................................................................................131
5 SUMMARY AND CONCLUSIONS ........................................................................................................138

REFERENCES ......................................................................................................................................141
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Endothelial Function</td>
<td>8</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>HLM estimates for change in diameter with shear rate</td>
<td>93</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>HLM estimates for effects of condition on change in diameter with shear rate</td>
<td>94</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>HLM estimates for change in diameter with shear rate</td>
<td>128</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>HLM estimates for change in diameter with steady-state vs. transient shear rate</td>
<td>129</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Flow characteristics post-ischemia</td>
<td>130</td>
</tr>
<tr>
<td>Figure 2.1: Anatomy of the arterial wall</td>
<td>.............................................................6</td>
<td></td>
</tr>
<tr>
<td>Figure 2.2: Haemodynamic Stress</td>
<td>.............................................................10</td>
<td></td>
</tr>
<tr>
<td>Figure 2.3: Synchronous (A) and asynchronous (B) shear- and circumferential – stress waveforms</td>
<td>.............................................................12</td>
<td></td>
</tr>
<tr>
<td>Figure 2.4: Determination of Shear rate</td>
<td>.............................................................13</td>
<td></td>
</tr>
<tr>
<td>Figure 2.5: Laminar (A) vs. turbulent (B) flow.</td>
<td>.............................................................16</td>
<td></td>
</tr>
<tr>
<td>Figure 2.6: Putative mechanisms of mechanotransduction.</td>
<td>.............................................................17</td>
<td></td>
</tr>
<tr>
<td>Figure 2.7: Nitric oxide (NO)-dependant dilation..</td>
<td>.............................................................24</td>
<td></td>
</tr>
<tr>
<td>Figure 2.8 Relative contributions of endothelium-derived vasodilatory factors in healthy (A) and diseased (B) arteries.</td>
<td>.............................................................27</td>
<td></td>
</tr>
<tr>
<td>Figure 2.9: Typical brachial artery blood velocity profile..</td>
<td>.............................................................29</td>
<td></td>
</tr>
<tr>
<td>Figure 2.10: Acceleration and steady shear components.</td>
<td>.............................................................31</td>
<td></td>
</tr>
<tr>
<td>Figure 2.11: High vs. low velocity acceleration.</td>
<td>.............................................................31</td>
<td></td>
</tr>
<tr>
<td>Figure 2.12: Flow profiles used for in vitro cell culture studies.</td>
<td>.............................................................33</td>
<td></td>
</tr>
<tr>
<td>Figure 2.13: Impulse vs. pulsatile flow .............................................................35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure 2.14: Velocity acceleration- vs. shear stress – dependant dilation .............................................................37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure 2.15: Determinants of velocity acceleration .............................................................39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure 2.16: Shear rate and diameter responses to 4 minutes ischemia .............................................................59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure 2.17: Ultrasound image showing duplex mode on the brachial .............................................................52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.18: Semi-automated diameter analysis ................................................................. 54

Figure 2.19: Diameter waveforms from three cardiac cycles ........................................... 54

Figure 2.20: Velocity acceleration calculation ................................................................. 60

Figure 3.1: Five protocol stages ..................................................................................... 94

Figure 3.2: Velocity acceleration calculation ................................................................. 95

Figure 3.3: Representative blood velocity waveforms .................................................... 111

Figure 3.4: Effects of condition on velocity acceleration ............................................... 112

Figure 3.5: Effects of velocity acceleration on diameter and shear rate change .......... 109

Figure 3.6: Effects of acceleration condition on flow-mediated dilation ....................... 110

Figure 4.1: Five protocol stages ..................................................................................... 131

Figure 4.2: Representative velocity waveforms ............................................................. 132

Figure 4.3: Mean diameters and shear rates ................................................................. 133

Figure 4.4: Mean shear rates plotted against peak diameters for transient and steady-state conditions ................................................................. 134

Figure 4.5: Within-subject variance explained by shear rate expression ...................... 135

Figure 4.6: HLM model estimates for shear rates regressed against peak diameters .... 136

Figure 4.7: Change in Reynold’s number and pulsatility index during reactive hyperemia .... 137
CHAPTER I

INTRODUCTION

The pathological complications of atherosclerosis, namely heart attacks and strokes, remain the leading cause of mortality in the Western world (8). Preceding atherosclerosis is endothelial dysfunction (48, 184, 190). The endothelium comprises a continuous monolayer of cells which separate the vascular wall from the circulation (128). Disruption of this essential monolayer is thought to occur early in the pathogenesis of cardiovascular disease. There is, therefore, interest in the application of clinical tools to non-invasively assess in vivo endothelial function. The flow-mediated dilation (FMD) test is the standard tool to non-invasively assess endothelial function (41). Reduced FMD is an early marker of atherosclerosis (41) and has been noted for its predictive capacity for future cardiovascular complications (155, 206). Despite its potential, the validity of FMD test has been questioned due to the lack of normalization to the primary stimulus (147, 181).

FMD is the increase in vessel diameter in response to increased blood velocity. The increase in velocity stimulates endothelial cell release of vasodilators, most notably nitric oxide, which result in smooth muscle cell relaxation (76, 107, 136, 151). Endothelial cells also release many other molecules which serve to maintain vascular homeostasis and prevent the instigation of atherosclerosis (136). A test has been developed which will be referred to as the FMD test (41). Typically, the FMD test is conducted on the brachial artery. Ultrasound is used to image the artery and measure dilatory responses to increased blood velocity. This test is accomplished by inflating a
cuff around the forearm to a supra-systolic pressure for 4.5-5 minutes (41). Release of the
cuff results in a large transient increase in blood velocity. The magnitude of FMD,
expressed as the percentage increase in diameter above rest, is used to represent
endothelial health

Despite the term flow-mediated dilatation, shear stress is the established stimulus
for FMD (120). Shear stress is determined by red blood cells moving close to the
endothelial cells. The magnitude of the shear stimulus created with reactive hyperemia is
influenced by several factors. Subsequently, the shear stimulus may differ significantly
between individuals. Therefore, in order to efficaciously compare groups of individuals,
the FMD test should be normalized to the shear stimulus. For instance, Mitchell et al.,
(147) demonstrated that reduced FMD may be attributable not only to impaired
endothelial release of nitric oxide, but also as a result of a lesser shear stimulus.
Fortunately, the ultrasound technology used to conduct the FMD test can also provide
estimates of shear stress. Shear stress can be calculated as the product of shear rate and
blood viscosity. Shear rate is typically estimated using the equation:

\[
\text{Shear rate} = \frac{8v}{d}
\]

where \( v \) is the mean flow velocity of blood, and \( d \) is the mean internal arterial
diameter.

The following discussion addresses two potential limitations inherent to
calculations of shear rate. 1) The assumption that mean blood velocity explains the shear
stimulus. And, 2) The transient nature of the shear stimulus used by the FMD test.
Due to the pulsatile nature of blood flow, mean blood velocity may not adequately define the shear stimulus. Endothelial cells are exposed to two distinct shear stimuli during the cardiac cycle: a large rate of change (velocity acceleration) in shear at the onset of flow (systole), followed by steady shear (diastole). In vitro studies suggest that these two distinct fluid stimuli regulate short- and long-term endothelial function via independent biomechanical pathways (68, 75, 122). Velocity acceleration may be an important independent stimulus for FMD. Conditions which affect velocity acceleration include ventricular ischemia (198), acute myocardial infarction (116), stenosis (20); hypertension (201); and hyperthyroidism (43). Velocity acceleration is also altered with aging (201) and physical activity (28, 175, 208).

While FMD is certainly attenuated in a number of disease states, FMD may also be “attenuated” if the hyperemic (shear stress) response is lower than expected. Peak- and time integrated-shear rates can vary substantially between-individuals (110, 127, 214). It is therefore imperative that the magnitude of the stimulus imposed on the endothelial cells is quantified. A previous study from our laboratory suggests that the shear stimulus is best quantified by integrating the hyperemic (shear) response (214). However, other groups suggest that the peak shear response is also important in determining FMD (110, 127). There remains a need to comprehensively quantify the appropriate expression of the shear stimulus.

**Study Significance**

Assessments of endothelial function offer the potential to predict and track individuals at risk for cardiovascular complications. Reliable FMD assessments are dependent on normalization to the stimulus, i.e. shear stress. It is hoped that better
quantification of the shear stimulus will lead to the development of a more sensitive and reliable FMD test. The current studies aims to identify the in vivo blood velocity parameters contributing to the FMD phenomenon. The FMD test *per se* is not measured. The shear rate-diameter relationship is assessed using ischemia, forearm heating, and handgrip exercise to indirectly increase shear rate through the brachial artery.

**Purpose Statement**

The purpose of study one was to determine the importance of velocity acceleration to FMD. FMD was measured prior to and following induced increases in velocity acceleration. Mean blood velocity was kept constant between conditions. The purpose of study two was to assess whether peak- and time integrated-shear rates independently predict FMD.

**Hypotheses**

*The hypothesis for study one (chapter 3) are:*

3.1. Increasing velocity acceleration does augment FMD.

*The hypothesis for study two (chapter 4) are:*

4.1. Peak shear rate does independently predict FMD.
CHAPTER II

LITERATURE REVIEW

This following review provides a comprehensive step-by-step overview of the shear stress stimuli regulating endothelial function. Summaries are given at important junctions throughout the review in order to tie together pertinent information. Finally, an overview is given to the requirements for non-invasive assessments endothelial function.

Anatomy and Functions of the Vascular Endothelium

Why Are Measurements of Endothelial Function Important?

Cardiovascular disease has a very long asymptomatic phase of development starting as early as the first decade of life. The Framingham Coronary Risk Score (with family history) (241) is a tested measure of risk; however, it can predict only 60% to 70% of cardiovascular events. In addition, there is a large population of patients who are at intermediate risk and are asymptomatic. The development of non-invasive tests of endothelial function could offer clinicians the capacity to screen individual patients who may be asymptomatic but who carry increased risk. The same screening tool could be used to track the benefits of subsequent targeted therapy.

The vascular endothelium performs many functions geared towards the maintenance of vascular homeostasis. Studies have shown that endothelial dysfunction is independently related to future cardiovascular events (e.g. myocardial infarction, stroke, transient ischemic attack) (87, 88, 92, 160, 204, 218, 246). An impaired vascular response
has also been demonstrated in children as young as 7 years old with familial hypercholesterolemia (213).

*The Vascular Endothelium*

From the lumen to the outer wall all arteries are composed of an intima, media, and adventitia (Figure 2.1). The intima is the inner most lining of the vessel, and comprises the endothelium and underlying connective tissue. The media is comprised mainly of vascular smooth muscle cells that regulate blood flow by vasoconstriction or vasodilation. The adventitia is the outer most layer and is mainly composed of connective tissue that maintains the shape of the vessels and limits distention.

![Figure 2.1. Anatomy of the arterial wall. (A) A conduit artery imaged in the longitudinal plane using ultrasound. (B) The layers comprising the wall of an artery. Endothelial cells form a continuous layer lining the intima throughout the arterial tree.](image-url)
Vascular endothelial cells essentially have the same characteristics as all the cells of the human body; cytoplasm and organelles surrounding a nucleus and contained by the cellular membrane. Endothelial cells form a continuous flat mono-layer of cells that cover the vascular lumina throughout the arterial tree. The endothelium is mechanically and metabolically strategically located, separating the vascular wall from the circulation and the blood components (128).

*Endothelial Cell Functions*

Endothelial cells perform autocrine, paracrine, and endocrine functions that serve to maintain vascular homeostasis (136). Endothelial cells are capable of producing a variety of agonistic and antagonistic molecules, including: vasodilators and vasoconstrictors, pro-coagulants and anti-coagulants, inflammatory and anti-inflammatory, fibrinolytics and anti-fibrinolytics, oxidizing and anti-oxidizing, and many others (Table 2.1) (136).

*Endothelial Dysfunction and Atherosclerosis*

Upsetting the delicate balance of functions performed by the endothelium (Table 2.1) initiates a number of events that promote atherosclerosis. Atherosclerosis is the precursor to cardiovascular disease. Although atherosclerosis is commonly described as the presence of plaques that obstruct the lumen of the conduit arteries, endothelial dysfunction precedes plaque formation (79, 162, 189). Reduced endothelial responses can be observed early in the course of atherogenesis, preceding angiographic or ultrasonic evidence of atherosclerotic plaque (136).
Table 2.1. *Endothelial unctions*.

<table>
<thead>
<tr>
<th>Role</th>
<th>Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular Tone</td>
<td>angiotensin II, endothelium-derived hyperpolarizing factor, endothelium-derived constricting factor, endothelin-1, <em>nitric oxide</em>, prostacyclin, prostaglandin H₂, thromboxane</td>
</tr>
<tr>
<td>Cell growth</td>
<td>angiotensin II, endothelin, heparan sulfate, <em>nitric oxide</em>, platelet derived growth factor, prostacyclin</td>
</tr>
<tr>
<td>Coagulation</td>
<td>dermatan sulfate, fibrinogen, heparin sulfate, <em>nitric oxide</em>, prostacyclin, thrombomodulin, tissue factor, thromboxane, von Willebrand’s factor</td>
</tr>
<tr>
<td>Erythrocyte adherence</td>
<td>Integrins</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>plasminogen activator inhibitor, tissue plasminogen activator</td>
</tr>
<tr>
<td>Inflammation</td>
<td>P and E Selectin, nuclear factor kappa beta, interleukin 8, and intracellular adhesion molecules, <em>nitric oxide</em>, vascular cellular adhesion molecules, receptor for advanced glycosylated end-products</td>
</tr>
<tr>
<td>Permeability</td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Ecto-ADPase⁹, <em>nitric oxide</em>, prostacyclin</td>
</tr>
<tr>
<td>Vasculogenesis/angiogenesis</td>
<td>platelet-derived growth factor, <em>nitric oxide</em>, transforming growth factor-beta, vascular endothelial growth factor</td>
</tr>
</tbody>
</table>

Note the multiple roles of nitric oxide and prostacyclin.

Disruption of the functional integrity of the vascular endothelium plays an integral role in all stages of atherogenesis ranging from lesion initiation to plaque rupture. Endothelial dysfunction leads to increased permeability to lipoproteins, foam cell formation, T-cell activation, and smooth muscle migration into the arterial wall (189). The first step in the formation of the plaque occurs when the inflammatory response is
incited and fatty streaks appear. If the situations persist, fatty streaks progress and the plaque are predisposed to rupture.

Summary

- The vascular endothelium maintains vascular homeostasis.
- Endothelial dysfunction initiates atherosclerosis
- Assessment of endothelial function can predict future cardiovascular disease events

Stimuli Regulating Endothelial Function

Various humoral stimuli, such as cytokines, growth factors, hormones, and metabolic products, can modulate endothelial cell function. Research over the past two decades has particularly emphasized the importance of haemodynamic conditions generated by the circulation of blood flow. The haemodynamic conditions inside blood vessels lead to the development of superficial stresses near the vessel walls (Figure 2.2), which can be divided into two categories: 1) circumferential stress due to pulse pressure variation inside the vessel and 2) shear stress due to blood flow (158, 173, 174).

Circumferential stress acts perpendicular to the vessel wall, whereas shear stress acts at a tangent to the wall to create a frictional force at the surface of the endothelium. Circumferential stress applies stress to all layers of the vessel wall (intima, media and adventia), while shear stress is applied principally at the endothelial surface. Although shear stress has a very small magnitude in comparison to circumferential stress, the endothelial cells are equipped with numerous mechanosensors to detect this stress. Shear stress is considered to be the primary stimulus regulating endothelial cell function (60).
Figure 2.2. *Haemodynamic stress.* Shear stress results in deformation in which parallel planes remain parallel but are shifted in a direction parallel to themselves, as opposed to normal stress or force, which when applied to an object induces normal (direct) deformation.

*Circumferential Stress*

As a result of pulsatile pressure changes, cells within the vascular wall are subjected to a three-dimensional cyclic strain in the radial, longitudinal and circumferential directions. Increases in transmural pressure generally appear to result in vasoconstriction in isolated perfused vessels (192). Likewise, exposure to pressure diminishes the production of the vasodilator nitric oxide by cultured endothelial cells (98). Increased transmural pressure has also been shown to decrease the vasodilatory response to acetylcholine, suggesting that pulsatile stretch may either attenuate the production of endothelium-derived vasodilatory factors or enhance that of endothelium-derived contracting factors (105, 191). This inhibitory effect on agonist-induced, nitric oxide-mediated dilation is even more pronounced in rhythmically stretched arterial segments (194). Conversely, perfusion of isolated vessels at differing flow rates, with
constant pressure, leads to vasodilation, which is reversed by cessation of flow or by inhibitors of nitric oxide synthase (49).

Most of the studies investigating the effects of circumferential stress on endothelial cells have done so using in vitro preparations. These studies typically assess circumferential stretch or shear stress in isolation. Although these studies have provided important information, the experimental conditions are substantially different from those in vivo. During each cardiac cycle, the ventricles eject a given volume of blood into the aorta and the pulmonary artery. At each site in the vasculature through which this pulse wave travels three basic wave phenomena can be observed: a pressure pulse, a flow pulse and a volume pulse (which is a change in cross-sectional area). Therefore, in vivo it is difficult to distinguish between pressure- and shear stress-induced changes in endothelial cell function because changes in circumferential stress and shear stress are inextricably connected (36, 182).

Several groups have found that the morphological and biochemical responses of endothelial cells simultaneously subjected to shear- and circumferential-stress differed from the response to the individual forces (21, 153, 248). Qui and Tarbell (182) found that the addition of circumferential stress to oscillatory shear stress enhances the production of vasodilators. However, they also found that the interaction between shear stress and circumferential stress is modulated by the degree of synchronization for circumferential stress and shear stress waveforms (Figure 2.3). Global wave reflection in the circulation and the local inertial effects of blood flow cause time lags between circumferential stretch and shear stress, resulting in a temporal phase angle -which has been termed the ‘stress phase angle’. When circumferential stress and shear stress are
out-of-phase this generates a complex, time-varying mechanical force pattern on the endothelial cell layer. Several studies have demonstrated large stress phase angles are pro-atherogenic and inhibit flow-induced release of vasodilators (nitric oxide & prostacyclin) (56-58, 126, 182, 183).

Figure 2.3. Synchronous (A) vs. asynchronous (B) shear and circumferential-stress waveforms. Synchronous (A) waveforms promote endothelial function. Asynchronous waveforms (B) promote endothelial dysfunction.

Shear Stress –The Primary Stimulus Regulating Endothelial Function

The magnitude of the shear stress to which the endothelial cell surface is subjected is given by the product of the dynamic viscosity of blood and shear rate. Viscosity is an internal property of a fluid that offers resistance to flow. For Newtonian fluids, shear rate and viscosity are directly related. However, the relationship between shear rate and viscosity is non-linear for non-Newtonian fluids. The determination of shear rate is based on the assumption of fluid mechanics in which the velocity of fluid
upon the surface is zero (no-slip condition). As the fluid particles “travel” parallel to the wall, their velocity increases from zero at the wall to a maximum value at some distance from the wall. This leads to the establishment of a gradient, which is defined as *shear rate* (Figure 2.4).

![Diagram](image)

**Figure 2.4. Determination of shear rate (γ).** Shear rate is determined by fluid particles traveling parallel to the vessel wall, their velocity increases from zero at the wall to a maximum value at some distance from the wall. The actual shear rate at the vessel wall is determined by shape of the velocity profile.

### Shear Stress Estimation

Most studies in humans have estimated shear stress by employing a simplified mathematical model based on Poiseuille’s law. Shear stress is calculated as the product of shear rate and blood viscosity, where shear rate equals:
Shear rate \( (\gamma) = \frac{2(2+n)v}{d} \)

where \( d \) is the internal arterial diameter, \( v \) is the time averaged mean blood velocity, and \( n \) represents the shape of the velocity profile. For a fully developed parabolic profile, \( n \) is 2 which is the normal assumption when estimating shear rate.

Poiseuille’s law assumes that: 1) the fluid (blood) is Newtonian, 2) blood flows through a rigid tube, 3) the velocity profile is parabolic, 4) whole blood viscosity represents viscosity at the vessel wall and is linearly proportional to shear rate, and, 5) mean blood velocity adequately defines the shear stimulus.

Although blood is a non-Newtonian fluid at low shear rates (smaller than approx 100 s\(^{-1}\)) (44) in vivo, shear rates in large arteries, particularly at the endothelial surface, are generally considerably larger than this threshold value so that the effect of the non-Newtonian behavior does not appear to be pronounced.

Blood vessels are distensible, meaning that increases in arterial cross-section occur during the cardiac cycle. Thus can reduce wall shear rate ~ 30% as compared with rigid tubes (67).

In arteries, the velocity profile will not develop to a full parabola as a consequence of flow unsteadiness and short vessel entrance lengths. In both arteries and arterioles, the velocity profiles are flattened parabolas (187). In the common carotid artery, mean wall shear stress is underestimated by a factor of 2 when assuming of a parabolic velocity profile (55). However, in the brachial artery the underestimation is
less pronounced, likely due to a more parabolic velocity profile in this artery, i.e. \( n \) is closer to 2 (55).

In order for the profile to be parabolic, blood flow also needs to be laminar, meaning that fluid is proposed to flow in layers. However, laminar flow may turn turbulent at high Reynolds' numbers \((Re)\):

\[
Re = \frac{vd\rho}{\eta}
\]

where \( v \) = peak systolic velocity, \( d \) = diameter, \( \rho \) = density, and \( \eta \) = viscosity.

Increase of flow velocity \((v)\), vessel diameter \((d)\) and fluid density \((\rho)\) increase \(Re\), while increase of viscosity \((\eta)\) has the opposite effect. Beyond the critical Reynolds number of 2,000, flow becomes turbulent, leading to a blunt flow profile. As a result Poiseuille’s law is no longer valid. Resistance to flow increases and therefore, a greater pressure difference is needed to produce an increase in flow rate compared with laminar conditions. Turbulent flow also results in complicated velocity gradients (Figure 2.5).

To estimate shear stress from shear rate, invasive measures of blood viscosity are required. Estimating shear stress from shear rate and viscosity potentially adds another source of error. Assessments of whole blood viscosity overestimate the viscosity at the wall of the vessel. The viscosity is higher in the center of the vessel, because red blood cells tend to stream in the center of the vessel, thereby reducing the shear stress gradients they are exposed to. Less red blood cells travel along the artery wall, where, in addition
to a thin layer of plasma, blood platelets are traveling. They are likely dispersed from the center of the vessel due to collision with the larger red blood cells (220).

Shear stress assessments do not seem to result in conclusions different from shear rate assessments alone. This may be explained by two factors: 1) sources of error from whole blood viscosity estimates, and, 2) for a given population viscosity changes little. Shear rate can therefore be used as an adequate surrogate measure (25, 81, 110, 180).

Mean blood velocity is unlikely to adequately characterize the shear stimulus. Due to the pulsatile nature of circulation, for a given mean blood velocity the flow profile can vary dramatically (14, 177, 178). Blood flow pulsatility results in endothelial cells being essentially exposed to two distinct shear stimuli during the cardiac cycle: a large rate of change (velocity acceleration) in shear at the onset of flow, followed by steady

Figure 2.5. *Laminar (A) vs. turbulent (B) flow*. Turbulent flow results in complex velocity gradients.
shear. In vitro studies suggest that these two distinct fluid stimuli regulate short- and long-term endothelial function via independent biomechanical pathways (11-13, 27, 101, 165, 238, 239). Velocity acceleration may be an important variable which interacts with mean blood velocity to govern the shear stimulus.

Non-Invasive Technology

The advent of ultrasound (32, 100) and magnetic resonance imaging (169) technologies has allowed the assessment of time-dependent velocities in human peripheral arteries. However, direct assessments of wall shear stress are not possible in vivo in humans. The limited resolution of ultrasound and magnetic resonance imaging disallows the capacity to measure blood velocity close to the vessel wall. Since shear rate is actually assessed at a distance from the wall, the values obtained have to be considered as least estimates. With predictable flow conditions shear stress values estimated at a distance from the artery wall will not differ significantly from those at the wall (~10%) (132). With magnetic resonance imaging, not only the spatial resolution, but also the temporal resolution is limited, the latter requiring special precautions to be able to study dynamic processes (78, 187). Magnetic resonance imaging also is expensive and not as readily available as ultrasound. Most in vivo studies have therefore adopted ultrasound technology.

Despite the limitations, shear stress assessments can be reliably made using ultrasound (202). Magnetic resonance imaging and especially ultrasound, both being non-invasive techniques, have provided valuable information about the levels of shear stress along the arterial tree in humans. However, the reliability of a measurement does not indicate whether the measurement is physiologically sound. Particularly during reactive
hyperemia, such as used to test FMD, the assumptions of Poiseuille’s law are likely violated and the accuracy of shear stress estimates will be abridged. Under these conditions mean blood velocity may not accurately characterize the shear stimulus. There is a transparent lack of non-invasive studies which assess the importance of the velocity profile to non-invasive estimates of shear stress.

**Shear Stress-Mediated Control of Endothelial Function**

*Shear Stress Mechanotransduction*

The endothelium is a complex mechanical signal-transduction interface between flowing blood and the vessel wall. Mechanotransduction is the interaction between shear stress-induced biomechanical and endothelial cell function. Exactly how these biomechanical forces are sensed by endothelial cells remains debated. Two models of mechanotransduction have been demonstrated so far.

*The localized model.* The mechanoreceptor, like other receptors, is considered to be located in the cell membrane (Figure 2.6). Channels (i.e. potassium, sodium and calcium) located in membrane respond to changes in shear stress. Because ion channel activation is one of the fastest known endothelial responses to flow, these ion channels have been proposed as the candidate flow sensors (15, 60, 71, 73, 123, 210). The type of flow-sensitive ion channel first identified in endothelial cells was an inward-rectifying K⁺ channel whose activation leads to cell membrane hyperpolarization (109, 166). A second type of flow-sensitive ion channel that has been more recently discovered is an outward-rectifying Cl⁻ channel. The change in membrane potential associated with the activation of these ion channels alters the electrochemical gradient for Ca^{2+} transport across the endothelial cell membrane. This provides a mechanism of direct interaction between
flow-sensitive ion channels and some of the Ca$^{2+}$-dependent second messenger pathways already described.

Figure 2.6. *Putative mechanisms of mechanotransduction.* The *localized* model for Shear stress mechanotransduction assumes that shear stress sensors are located in the cell membrane. Ion channels have been proposed as the candidate flow sensors. The decentralized *model* suggests that the mechanical forces acting on the luminal side of the endothelial cells are transmitted through the cytoskeleton to other sites in the cell. Integrins connected to the cytoskeleton have been related to this mechanism of mechanoreception.

The Cl$^{-}$ and K$^{+}$ channels are activated independently (16, 157). Cl$^{-}$ activation leads to cell membrane depolarization following the initial K$^{+}$ channel-mediated hyperpolarization. The fact that hyperpolarization precedes depolarization in spite of the larger electrochemical driving force for Cl$^{-}$ than K$^{+}$ suggests that flow-sensitive Cl$^{-}$ channels attain maximal activation more slowly than flow-sensitive K$^{+}$ channels. The notion that K$^{+}$ channels respond to shear stress more rapidly than Cl$^{-}$ channels is expected to be particularly relevant for situations where a time-varying shear stress may activate...
one or both channels depending on the time constant characterizing the changes in shear stress.

There is mounting evidence that flow-sensitive $K^+$ and Cl$^-$ channels play a central role in regulating overall endothelial responsiveness to flow. This construct is supported by data demonstrating that interference with these candidate mechanosensors affects downstream gene and protein regulatory responses. For instance, pharmacological antagonists of flow sensitive $K^+$ and Cl$^-$ channels greatly attenuate or entirely abolish shear stress–induced release of cyclic guanosine monophosphate (49) and nitric oxide, down-regulation of endothelin-1 (139) up-regulation of transforming growth factor-beta mRNA (76), and induction of Na-K-Cl co-transport protein (217). Furthermore, interfering with G-proteins also abolishes several endothelial cell flow responses including release of nitric oxide (57) and prostacyclin (91) as well as flow-stimulated increases in mitogen-activated protein kinase activity (61).

The “decentralised model”. This model suggests that the mechanical forces acting on the luminal side of the endothelial cells are transmitted through the cytoskeleton to other sites in the cell (93). The endothelial cell can be viewed as a membrane stretched over a frame composed of intermediate filaments and actin fibers which transverse the cells and end in adhesion complexes (Figure 2.6). Even under non-stimulated conditions, the entire endothelial cytoskeleton is maintained under tension and in response to an externally applied stimulus intracellular tension is redistributed over the cytoskeleton network. These forces are especially sensed at the basal adhesion points, where the endothelial cell is attached to the extracellular matrix, cell junctions and the nuclear membrane (60). So it is conceivable that the application of a stress activates
signal transduction cascades without the need of a specific shear stress or stretch receptor. Integrins connected to the cytoskeleton have been related to this mechanism of mechanoreception (235).

**Endothelial Responses to Shear Stress**

In response to mechanotransduced shear stress, endothelial cells produce short to intermediate term acting agents and proteins or long term activated expression of many genes inducing complete cell remodeling.

*Short-Term.* Acute exposure to flow is defined as changes in flow that last from a few seconds up to a few hours. To maintain physiological levels of vessel wall shear stress and flow rate, vascular tissues respond to changes in shear stress with acute adjustments in vascular tone and with chronic structural remodeling when variations in shear stress persist (125). Large blood vessels, such as elastic and muscular arteries, can undergo dramatic adaptations in response to both acute and chronic alterations in blood flow that appear to be endothelium-dependent. Some of these changes, such as vasodilation and vasoconstriction, reflect alterations in the rates of production of endothelial-derived mediators, such as prostacyclin and nitric oxide, which act locally to modulate the tone of vascular smooth muscle. Typically, this level of regulation involves changes in the availability of substrate or the activity of rate-limiting enzymes, and appears to serve a homeostatic function in maintaining constancy of tissue perfusion in response to fluctuating metabolic demands. The endothelium performs these functions by regulating the expression of critical vasoactive and growth factors such as endothelin-1, nitric oxide synthases, and platelet-derived growth factor A and B chains (12, 68,69).
Long-term. Chronic changes in blood flow can elicit more profound structural remodeling involving cell proliferation and cell death (e.g., apoptosis), as well as hypertrophy of extracellular matrix. These longer-term structural modifications reflect a complex balance of locally generated growth inhibitory and growth stimulatory substances which are endothelium-dependent. The magnitude of chronic shear stress also influences the expression of genes that have a protective role against atherosclerosis such as transforming growth factor beta-1 (135) as well as genes that have been implicated in the development of atherosclerotic lesions such as the adhesion molecules intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 (156, 226).

Summary

- Shear stress induces a wide variety short- and long-term endothelial cell responses.
- Endothelial cells may sense change in shear stress via localized ion channels located in the cell membrane.
- Alternatively, shear stress mechanotransduction may be decentralized. Mechanical forces may transmit signals through the cytoskeleton to other sites in the cell.

Flow-Mediated Dilation

In 1970, Rodbard (188) proposed that the endothelium may sense and respond to the shear stress generated by flowing blood. In 1980, Furchgott and Zawadski (76) discovered that agonist-mediated vasodilation requires participation by the endothelium. The dependence of FMD on an intact endothelium was subsequently shown to occur in
large-conduit arteries as well as in resistance-sized vessels (193). More recent studies have demonstrated that vasodilation is directly proportional to increasing shear stress (120, 151). Endothelial cells induce vasodilation by releasing vasodilatory factors, including nitric oxide, prostacyclin, and a putative endothelium derived hyperpolarizing factor.

**Nitric Oxide**

In 1986, Furchgott and Ignarro *et al.* (107) proposed that the endothelial mediator involved in endothelium-dependent relaxation is nitric oxide. Moncada and coworkers (171, 172) confirmed that indeed nitric oxide is a major endothelium-derived relaxing factor. By providing the scientific community with inhibitors of nitric oxide synthase (NOS) (185, 186) they made the exploration of the physiological role of nitric oxide possible. Nitric oxide is a biologically active and volatile gas that is present practically in all tissue. Due to its low molecular weight and its lipophilic properties it diffuses easily across cell membranes.

In response to a number of physical (e.g., flow/shear stress) and chemical (e.g., acetylcholine) stimuli, cell signaling events lead to an elevation in endothelial cell cystolic Ca$^{2+}$, which binds to the regulatory protein calmodulin (37) (Figure 2.7). Calcium displaces the inhibitor caveolin-1 from calmodulin allowing Ca$^{2+}$-calmodulin to activate endothelial NOS (eNOS). The enzymatic actions of eNOS lead to nitric oxide being formed in endothelial cells from its precursor, L-arginine (18). Nitric oxide diffuses across the endothelial intima and reaches the smooth muscle cells where it stimulates guanylate cyclase-catalyzed production of cyclic guanosine monophosphate (70, 232). Cyclic guanosine monophosphate down-regulates the contractile process by antagonizing
calcium release from the sarcoplasmic reticulum, its entry through calcium channels, and by inhibiting the calcium activated contractile process itself(131, 133).

Figure 2.7. Nitric oxide (NO)-dependent dilation. Shear stress initiates increased intracellular calcium (Ca\(^{2+}\)) which displace the inhibitor cavelon from calmodulin (CaM), activating eNOS. Nitric oxide diffuses to the smooth muscle, activates guanylate cyclase (GC, thereby increasing intracellular cyclic guanosine monophosphate (cGMP).

While Ca\(^{2+}\)-calmodulin-dependent eNOS activation is likely the primary pathway responsible for nitric oxide production, other pathways do exist. For example, in the pig coronary artery endothelium-dependent relaxation is only partially or not affected by blocking the increase in cyclic guanosine monophosphate with a NOS inhibitor(50). Nitric oxide can also be produced by isoforms of NOS other than eNOS. Studies show that nitric oxide released from neuronal NOS (nNOS) localized in neurons lining coronary and pial arteries can mediate flow- and agonist-induced dilations in eNOS knockout mice (103, 124, 145). This suggests a compensatory interaction between eNOS
and nNOS that could offset eNOS deficiencies. However, more work is required to clarify the relative role of nNOS-derived nitric oxide in vasomotor control in vessels with competent eNOS.

Prostacyclin

Flow-induced prostacyclin changes were one of the earliest documented responses of endothelial cells to shear stress (84). Prostacyclin is synthesized by cyclooxygenase and released from the endothelium (Figure 2.7); prostacyclin elicits smooth muscle relaxation by stimulating adenylyl cyclase and generation of cyclic adenosine monophosphate. Its half-life is longer than nitric oxide (~3 minutes vs. a few seconds for nitric oxide).

Endothelium-Derived Hyperpolarizing Factor.

In the late 1980s and early 1990s, evidence surfaced that suggested at least one additional endothelium-dependent process responsible for relaxing vascular smooth muscle in addition to nitric oxide and prostacyclin. The process was characterized by an essential hyperpolarization of the vascular smooth muscle and could be blocked by inhibitors of potassium channels. The mediator became known as endothelium-derived hyperpolarizing factor (EDHF) (33, 222). Unlike its predecessor, endothelium-derived relaxing factor, which required approximately 6 years before conclusively being identified as nitric oxide, the mechanism[s] of EDHF remains controversial even today (33). The elusive nature of EDHF is likely due to the fact that there are several EDHFs or mechanisms by which the endothelium can hyperpolarize vascular smooth muscle (33).
Endothelium-derived hyperpolarizing factor is defined as a dilator that: 1) requires endothelium, 2) is distinct from both endothelium-derived nitric oxide or cyclooxygenase metabolites (i.e., prostacyclin), 3) dilates by hyperpolarizing the vascular smooth muscle, and 4) involves potassium channel activation, most often calcium-activated potassium channels (82). EDHF dilations can be elicited by a number of endothelial agonists, shear stress, or pulsatile stretch (33, 77, 82, 104, 179, 219).

Relative Contributions of Nitric Oxide, Prostacyclin & EDHF

Osani et al. (168) showed that inhibition of eNOS enhanced flow-induced production of prostacyclin, indicating that endogenous nitric functions as an inhibitor of prostacyclin production in an autocrine or paracrine fashion. Other studies have reported similar findings (17, 26, 142) (118). In vivo, clinical studies have shown that nitric oxide production was impaired in patients with atherosclerosis (106), whereas prostacyclin production was not reduced (72). The possibility arises that vessel homeostasis may be maintained through a compensatory increase in prostacyclin in vivo when the output of nitric oxide is decreased.

The degree to which EDHF contributes to relaxation may be small or nonexistent. An estimate of a 20% to 30% contribution of hyperpolarization to relaxation was made by comparing the hyperpolarization and relaxation induced in rat aorta by acetylcholine and a potassium channel opener (BRL38227) (165). It is possible that nitric oxide inhibits the formation or action of EDHF in normal, healthy vessels (47). However, in diseased vessels reductions in nitric oxide bioavailability may indirectly up-regulate EDHF. In fact, EDHF has been reported to be up-regulated after a variety of pathologic conditions when nitric oxide-mediated dilations have been attenuated. The up-
regulation seems to occur after ischemia–reperfusion, traumatic injury, congestive heart failure, coronary artery disease, hypercholesterolemia, and angioplasty (31, 83, 140, 144, 149, 224). In those pathologic conditions where EDHF is up-regulated, it is thought to be a protective mechanism that compensates for insufficient endothelium-derived nitric oxide.

Figure 2.8. Relative contributions of endothelium-derived vasodilatory factors in healthy (A) and diseased (B) arteries. While nitric oxide (NO) is the primary vasodilatory in healthy arteries, the relative importance of prostacyclin (PGI₂) and endothelium-derived hyperpolarizing (EDHF) factor appears to be increased for diseased arteries. Improved understanding of the compensatory mechanisms may lead to new therapeutic targets.

Summary

- Endothelial cells induce FMD by releasing dilatory molecules, including nitric oxide, prostacyclin & EDHF.
- The relative contributions of these molecules to FMD may be altered by disease states.
Influence of the Blood Flow Profile on Endothelial Responses

The earliest studies investigating the implications of shear stress on endothelial function did so by assessing endothelial cell responses to high versus low shear stress (39). This was until Davies et al. (62) in 1986 that provided evidence that the time-averaged shear stress alone could not explain the pathological behavior of endothelial cells exposed to the complex flow patterns. Subsequent studies (7, 14, 27, 53, 95, 129, 135, 143, 176, 229, 236) have shown that vascular endothelial cells respond not only to the time averaged shear stress, but respond differently to different patterns of flow. The following sections will provide an insight into the nature of circulation, followed by an overview of endothelial cell responses to different types of flow paradigms.

Circulation

The cyclic nature of the beating heart creates pulsatile conditions in all arteries. The heart pumps out blood during systole and rests during diastole. These cyclic conditions create relatively simple mono-phasic flow pulses in the upper region of aorta (244). However, pressure and flow characteristics are substantially altered as blood circulates through the arterial tree. Figure 2.9 shows an example of a typical brachial artery blood velocity profile taken using Doppler ultrasound. The normal brachial arterial signal is tri-phasic, corresponding to the: 1) rapid blood flow during systole, 2) initial reversal of blood flow in diastole, and, (3) gradual return of forward flow during the late phase of diastole.

The blood flow profile in the aorta will be predominately governed by the force of blood ejected from the heart (234). However, in the periphery, the blood flow profile becomes more complex as a result of the energy transfer between the heart and arteries.
The heart generates forward-traveling wave energy that propagates through the arteries to maintain tissue and organ perfusion for metabolic homeostasis. An individual forward-traveling waveform, generated by the heart at the beginning of cardiac systole, initiates flow and increases pressure in the arteries. Although most of the wave energy in this initial compression wave travels distally into smaller arteries, some is reflected back toward the heart at sites of impedance mismatch (34). Interactions between forward- and backward-traveling waves result in complex patterns of blood flow and pressure change at different points in the arterial circulation. Wave reflections result from arterial geometry, arterial wall compliance, and downstream resistance created resistance arteries (14, 177, 178).

Figure 2.9. Typical brachial artery blood velocity profile. The thick horizontal line represents a velocity of ‘0’. Note the presence of reverse flow during diastole.
Complex flow characteristics have a profound impact on the shear stress distribution to which vascular endothelial cells are exposed. While, human in vivo experiments typically denote shear stress as a mean construct, numerous secondary phenomena associated with flow, including pulsatile flow, reversing flow, and turbulence, can influence the regulation of endothelial cells.

Velocity Acceleration and Endothelial Function

The pulsatile nature of blood flow results in endothelial cells being exposed to two distinct shear stimuli during the cardiac cycle: a large rate of change in shear at the onset of flow, followed by steady flow (Figure 2.10). The large rate of change in shear results in temporal shear gradients. Temporal shear stress gradients are defined as the increase or decrease of shear stress over a small period of time at the same location. Temporal shear gradients are a second derivative of blood velocity. In vivo, we measure systolic velocity acceleration as a surrogate marker for temporal gradients in shear stress. The term velocity acceleration will thus be used to incorporate temporal gradients in shear stress. In vitro studies suggest that these two distinct fluid stimuli (velocity acceleration vs. steady velocity) regulate short- and long-term endothelial function via independent biomechanical pathways (11-13, 27, 101, 165, 238, 239). For a given mean blood velocity, or shear stress, velocity acceleration can vary quite substantially (Figure 2.10). These studies have also that the rate of velocity acceleration can affect the progression of atherosclerosis, endothelial cell function, mechanotransduction, calcium kinetics, and vascular tone. The following section summarizes major findings, followed by an overview the in vivo human studies which have indirectly shown that altering velocity acceleration may have athero-protective effects.
Figure 2.10. *Acceleration and steady shear components.*

Figure 2.11. *High vs. low velocity acceleration (VA).* The horizontal line denotes identical mean shear stress for both VA rates.

*In vitro flow profiles.* In vitro studies offer the advantages of being able to manipulate flow profiles in order to isolate the effects of velocity acceleration on a
number of endothelial cell function markers (Figure 2.13). A brief description of the different flow profiles utilized is important in order to elucidate which specific aspects of the flow profile regulate endothelial cell function. An understanding of these flow models will also help to explicate why the literature has produced some discrepant findings.

Figure 2.12 gives a simplified overview of the velocity profiles which have been employed. Earlier studies generally utilized either ramp flow (flow smoothly transitioned to steady state) or step flow (flow abruptly increased to steady state) models. Ramp flow results in low velocity acceleration. Step flow results in high velocity acceleration. Others studies have utilized impulse flow (flow abruptly applied for ~0.5-3 s only) or periodic flow (repeated impulses) models to determine the effects of repeated changes in velocity acceleration. More recent studies have used pulsatile flow models to more accurately mimic the in vivo environment that endothelial cells are exposed to. Pulsatile flow incorporates sharp changes in flow (velocity acceleration) such as occurs during systole, followed by more steady flow, such as occurs during diastole. Oscillating flow has been used to mimic rapidly changing flow profiles which are devoid of a steady flow component. Reverse oscillating flow (abrupt periodic changes in flow direction) has been utilized to determine the importance of flow direction in governing the effects of velocity acceleration on endothelial cell function. It is important to note that these flow profiles are generally laminar, though a limited number of studies have utilized turbulent blood flow profiles.
Figure 2.12. *Flow profiles used for in vitro cell culture studies.*

*Atherosclerosis.* The effects of velocity acceleration on the development of atherosclerosis have produced conflicting findings. Bao et al. (13) found that velocity acceleration up-regulates the expression of putative atherogenic genes (monocyte chemoattractant protein-1 and platelet-derived growth factor-A) which are believed to participate in the early events of atherosclerosis (54, 240). Similarly, the same group found that velocity acceleration up-regulates endothelial cell proliferation (238). However, a more recent study by Hsiai et al. (102) found that pulsatile flow actually down-regulated the expression of monocyte chemoattractant protein-1 and reduced monocyte binding to lipid oxidized endothelial cells. Furthermore, they found that the effects were more exaggerated for pulsatile flow with high velocity acceleration vs. low velocity acceleration, even though mean shear stress was equivalent (50 dyne/cm²).
Apart from the use of different endothelial cell cultures, bovine aortic (13) vs. human umbilical (102), a fundamental difference between the two aforementioned studies lies in the flow paradigms utilized (Figure 2.13). Bao et al. (13) evoked a single flow impulse (abrupt 0 to 16 dynes/cm² sustained for 3 seconds), whereas Hsiai et al. (102), evoked pulsatile flow with a mean shear stress of 50 dyne/cm². Notably, the Hsiai et al. (102) flow model did not permit shear stress to return to zero between flow impulses, thereby, inducing a steady flow component. However, when Hsiai et al. (102) produced an oscillating flow profile (0 ± 5 dyne/cm²), which induces high velocity acceleration but has a mean shear stress of 0 dyne/cm² and is devoid of a steady flow component, monocyte chemoattractant protein-1 expression was up-regulated and monocyte binding was increased. This is consistent with the notion that while endothelial cells derive directional cues from the flow direction or velocity, a certain persistence of the stimulus is required (61, 94). Taken together, these findings suggest that endothelial cells are regulated by a complex interplay between the steady flow/shear stress and velocity acceleration.

In vivo, flow is pulsatile everywhere in the arterial system, but in most places there is a large steady component. However, at sites where flow oscillates with a low steady component atherosclerotic lesions are known to occur. This is consistent with the notion that while endothelial cells derive directional cues from the flow direction or velocity acceleration, a certain persistence of the stimulus is required (61, 94). Steady shear stress results in the continuous up-regulation of anti-atherogenic genes (manganese superoxide dismutase, cyclooxygenase-2, eNOS) (225), and promotes endothelial cell release of various bioactive substances that may be involved in the regulation of
atherogenic genes, such as nitric oxide (121, 152). Taken together, these findings suggest that the production of atherogenic genes by endothelial cells is regulated by a complex interplay between velocity acceleration and steady components of flow. These findings may also explain why step flow, which includes velocity acceleration followed by steady shear, does not evoke the same endothelial response as velocity acceleration (i.e. impulse flow) alone (13, 238).

Figure 2.13. **Impulse** (13) vs. **pulsatile flow** (102). Bao et al. (13) found velocity acceleration induced by impulse flow to up-regulate the expression of putative atherogenic genes (monocyte chemoattractant protein-1 and platelet-derived growth factor-A). Hsiai et al. (102) found velocity acceleration induced by pulsatile flow to down-regulate the expression of a putative atherogenic gene (monocyte chemoattractant protein-1). The discrepant findings may be explained by the different flow profiles between studies.

**Vasodilation.** The endothelium can mediate flow-mediated vasodilation by altering the release of numerous factors, including nitric oxide and prostacyclin. The
development of more sophisticated in vitro flow models has progressively allowed the effects of velocity acceleration on cultured endothelial cell function to be studied. Notably, the release of nitric oxide and prostacyclin from cultured endothelial cells has been directly related to the rate of velocity acceleration (74, 75, 122, 163). The rate of velocity acceleration has also been directly related to vasodilation of isolated cremaster arterioles (38).

The production rate of nitric oxide and prostacyclin following flow onset exhibits a biphasic response, an initial transient burst followed by a slower reduction to a constant rate (74, 75, 122). Separate studies from the same group (75, 122) demonstrated that the initial burst of nitric oxide is dependent on velocity acceleration but not shear stress magnitude. The subsequent constant nitric oxide production is dependent on shear magnitude. The velocity acceleration dependent burst is consistent with previous in vitro observations (24, 84) for flow-mediated release of prostacyclin and for the stimulation of nitric oxide release by Ca$^{2+}$ mobilizing agonists (134). Similar findings have also been observed in isolated perfused vessels exposed to an acute change in flow (38, 49, 192).

The velocity acceleration-dependent initial burst of nitric oxide and the subsequent constant nitric oxide production have been demonstrated to be modulated by different pathways (68, 75, 122) (Figure 2.14). Two studies from the same group (75, 122) found the initial burst of nitric oxide to be Ca$^{2+}$ and G-protein-dependent. In contrast, the subsequent constant nitric oxide production was found to be Ca$^{2+}$ and G-protein-independent. A recent study found that the initial velocity acceleration mechanotransduction signal for nitric oxide release may be dependent on
platelet/endothelial cell adhesion molecule-1 (PECAM-1) (68). PECAM-1, which acts as an intracellular bridge between the two plasma membranes of neighboring cells is complexed with eNOS at the cell-cell junction. Shear stress is thought to deform the endothelial cell plasma membrane and activate PCAM-1 (117). Dusserre et al. (68) found that the eNOS/PECAM-1 complex was activated by velocity acceleration but not shear stress *per se*.

![Diagram](image)

**Figure 2.14.** *Velocity acceleration vs. shear stress dependent dilation.* Velocity acceleration (VA) and mean shear stress per se appear to regulate nitric oxide production via distinct pathways.

The above findings potentially have important clinic implications. Agonists are often used to study in vivo human endothelial function. Similar to acute changes in flow, agonist-mediated nitric oxide production is a Ca$^{2+}$-calmodulin-dependent process (74, 134). Continued exposure to flow, i.e. a typical in vivo haemodynamic environment,
results in Ca\textsuperscript{2+}-calmodulin independent nitric oxide production. Therefore, agonist-mediated nitric oxide production may not give a complete picture of the capacity of \textit{in vivo} endothelial cells to regulate vascular tone.

\textit{Determinants of velocity acceleration.} Velocity acceleration reflects the force of blood flow, which is met with an equal opposing force created by the downstream resistance (Figure 2.15). The force of the blood flow is largely governed by myocardial contractility, explaining why velocity acceleration is manipulated by inotropic agents (22, 164, 233) but not changes in after-load or preload (211). Conversely, velocity acceleration is reduced by beta blockers (43) ventricular ischemia (198), acute myocardial infarction (116), and is directly correlated with ejection fraction (197). Decreased myocardial contractility may explain also explain why lower velocity acceleration was found at rest and during exercise for older subjects, even though stroke volume and cardiac output responses showed no significant age dependence (108). A strong positive correlation ($r = 0.79$, $P = 0.0008$) has been found between brachial artery velocity acceleration and aortic artery velocity acceleration (42), suggesting that myocardial contractility has a systemic velocity acceleration effect.

Resistance to flow is principally supplied by distal arterial beds (34, 89, 90). The effect of distal resistance on flow velocity is different during systole versus diastole. Arteries that supply a low-resistance vascular bed usually demonstrate lower systolic flow and high diastolic flow (90). Arterial flow to the high-resistance peripheral circulation usually is characterized by rapid acceleration in early systole, decreased duration of systole, and reversal of flow in early diastole (90). Distal resistance results in pressure waves being reflected and subsequently multi-phasic diastolic flow patterns
(40). When vasomotor tone is diminished (i.e., by warming the extremity), the reversed flow components may be absent.

Finally, flow resistance is also modulated by arterial compliance (34). As compliance increases velocity acceleration decreases, likely due to decreased pressure wave reflection (40). More compliant vessels are able to store a greater portion of kinetic energy from each systolic pulse (89). Compliance also modulates the duration of systolic flow and the magnitude of diastolic flow. The kinetic energy stored by compliant vessels is released at the end of systole, prolonging systolic flow and providing a greater drive for antegrade flow during diastole(89).

To summarize, aortic flow patterns are predominantly determined by myocardial contractility, whereas flow profiles observed throughout the vascular tree are more complex. The more complex flow patterns are the result of the flow – induced pressure wave interacting with the distal vascular resistance and with vascular compliance.
Importance of velocity acceleration in vivo. Velocity acceleration is altered by diseased states. Decreased velocity acceleration is seen with ventricular ischemia (198), acute myocardial infarction (116), and stenoses (20); increased velocity acceleration occurs with hypertension (201), hyperthyroidism (43), bypass grafts (9) and obstruction of the lumen (138). Velocity acceleration is also decreased by aging (201), and increased with physical activity (28, 175, 208) and vascular resistance (52, 201, 212). Inadequate definition of the shear stimulus may hinder comparisons between the aforementioned patient groups who exhibit differing rates of velocity acceleration, or even complicate observations on individuals undergoing therapy which may alter velocity acceleration.

Potential limitations of in vitro studies The relevance of findings garnered from in vitro studies bear two important limitations. Firstly, endothelial cells from different tissue beds have been shown to exhibit different responses for a given flow paradigm (10). For instance, it has been shown that different tissue beds exhibit different optimal flow frequencies for proliferation, eNOS activity, and prostacyclin secretion (10). 2) Secondly, most culture studies have investigated endothelial gene regulation by haemodynamic forces to a single type of stimulus (continuous laminar shear stress) applied to cultured endothelial monolayers. Therefore, although these simple in vitro model systems have yielded much valuable new information, they fail to mimic the complexities of in vivo environment. The applicability of these findings to humans remains to be determined.

Summary

- Complex flow characteristics have a profound impact on the shear stress distribution to which vascular endothelial cells are exposed.
• In particular, pulsatile flow results in endothelial cells being exposed to sharp shear stress gradients (velocity acceleration) with the onset of the cardiac cycle.
• Mean blood velocity may not adequately characterize the shear stimulus.

**FMD with Transient vs. Steady-State Shear Stress**

A limited number of studies have reported enhanced dilatory responses to steady state versus transient increases in flow (111, 154, 180). Pyke *et al.* (180) induced steady state increases in shear rate using forearm heating, and transient increases in shear using standard 5 minutes ischemia. Brachial artery shear rate was increased to 47s\(^{-1}\) under the steady state condition versus 97s\(^{-1}\) (average for 60s post cuff release) for the transient condition. Diameters increased by 0.15 mm versus 0.27 mm for the steady state versus transient conditions, respectively. Normalized to average shear rates, vasodilation was 13.5% greater for the steady state condition. Mullen *et al.* (154) similarly induced flow responses using hand heating and ischemia in the radial artery. Normalized to the average flow response, vasodilation was 60% greater under steady state versus transient conditions.

**Summary**

The duration of the shear stimulus appears an important governor of vasodilation. Differences in FMD to flow may be explained by: 1) shear stimulus over-estimation for transient conditions, or 2) differences in vasodilatory mechanisms induced by transient and steady-state conditions. The potential for sources of error being introduced by simplified estimates of shear rate has already been discussed. Non-invasively, shear rate is estimated based on Poiseuille’s law. Reactive hyperemia likely results in the
assumptions of Poiseuille’s law being violated. In the following section, the potential for different mechanistic pathways being recruited with steady state versus transient increases in shear is discussed.

**Vasodilatory Mechanisms for Steady-State vs. Transient Shear Stress**

The magnitude of hyperemia is dependent on the method used as well as subject characteristics. Reactive hyperemia has been evoked using different occlusion durations, cuff positions, and ischemia with the addition of forearm exercise (25, 110, 127, 154). Other studies have increased shear stress in the feeding conduit artery via hand warming, or infusing the vasodilator, acetylcholine, into the forearm circulation (111, 154). These latter techniques result in a shear stress profile that increases gradually and reaches a steady plateau. This is in sharp contrast to the large transient profile created with reactive hyperemia. Furthermore, the magnitude of hyperemia may differ between persons due to difference in: artery size, age, or sex. Hyperemic magnitude has also been shown to differ in patient groups.

Forearm exercise and heating have both been used to evoke steady local increases in flow. Typically, the arterial segment of interest (e.g. brachial artery) is upstream from the heated skin or the metabolically active muscle bed. These conditions drastically differ from ischemia-induced increases in flow, where the hyperemic response is very transient. Whereas ischemia-induced hyperemia has been demonstrated to result in nitric oxide-mediated vasodilation, the pathways governing vasodilation under steady-state conditions are less clear.

The endothelium is still thought to primarily govern vasodilation under steady-state conditions. Studies (110, 154, 180) have shown that hand warming has no effect on
brachial artery diameter when flow was not allowed to rise. Similarly, when radial artery blood flow is maintained at basal levels during hand warming, there was no significant dilatation of the radial artery. Furthermore, pharmacological blockade of the autonomic nervous system has no effect on radial artery FMD in response to hand warming, consistent with animal studies showing that FMD is preserved after surgical or pharmacological denervation (97, 130).

The idea that endothelial cells can promote vasodilatation through the release of several vasoactive substances is not a new one. Micro-vessels and cultured cells may release nitric oxide (119), prostacyclin (120), EDHF (35), and acetylcholine (141) in response to shear stress elevations. The relative importance of these vasodilators is dependent on the duration of the shear stimulus

*Nitric oxide.* Nitric oxide production is biphasic following the onset of laminar flow with an initial rapid rise at the onset of flow followed by a slow sustained increased level of production (121). The initial rapid production of nitric oxide is independent of shear stress magnitude, whereas the sustained release phase is shear dependent. With the use of selective inhibitors, the initial phase has been shown to be Ca\(^{2+}\)-calmodulin dependent in contrast to the sustained phase, which appeared to be independent of these messengers. Endothelial cells may contain both a Ca\(^{2+}\) -independent and a Ca\(^{2+}\) – dependent NOS, each of which may be under multiple controls for activation by shear stress or by agonists (137).

*Prostacyclin.* The prostacyclin response is also biphasic. After an initial rapid release, production declines for several hours before recovering to maintain a steady
release rate. The second phase depends on an exogenous source of arachidonic acid and is directly related to the magnitude of the shear stress (84).

**Human studies.** Mullen *et al.* (154) found that radial artery FMD in response to a brief shear stress stimulus created with 5 minutes of wrist occlusion was abolished with the infusion of a NOS inhibitor (L-NMMA), indicating nitric oxide dependence. They also administered 15 minutes occlusion, which resulted in the same peak but a more prolonged hyperemia. FMD was not affected by inhibition of nitric oxide or prostacyclin pathways. Furthermore, when they created a very prolonged, steady increase in shear stress with hand warming or distal acetylcholine infusion, the resultant FMD was similarly unaffected by inhibition of nitric oxide or prostacyclin. These results indicate that the FMD response to prolonged increases in shear stress is not mediated by Ca$^{2+}$-calmodulin-eNOS-dependent nitric oxide pathways or prostacyclin.

Distal versus proximal cuff position also affects the duration of reactive hyperemia. Proximal occlusion results in a greater reactive hyperemic duration compared to distal occlusion, and therefore a greater stimulus (5, 23, 25, 64). Doshi *et al.* (64) demonstrated that while the FMD in the brachial artery in response to distal occlusion-induced reactive hyperemia was abolished with NOS inhibition, the response to proximal occlusion was only partially attenuated from ~12% to ~8%. However, proximal occlusion also introduces other confounding factors since the area of measurement is itself ischemic and undergoes a dramatic pressure change with occlusion.

**Redundant mechanisms.** A number of studies have found that NOS inhibition (with L-NAME or L-NMMA,) reduces blood flow responses ~20-30% with forearm handgrip exercise (65, 66, 69, 80, 85, 113). Prostacyclin inhibition has also been shown
to reduce blood flow response to forearm exercise (65, 66, 205) demonstrating a role for both nitric oxide and prostacyclin in mediating hyperemia in response to exercise. Interestingly, sequential inhibition of these vasodilators was found to have no additional effect on reducing blood flow above that seen with the inhibition of one. This was confirmed by Schrage et al. (30), who assessed brachial artery blood flow using Doppler ultrasound. Blood flow reductions with nitric oxide or prostacyclin inhibition were not additive.

Although the precise interaction between FMD mechanisms with a prolonged shear stress stimulus remains unclear, the above observations suggest that the mechanisms primarily responsible for the observed FMD response may change over time when the shear stress stimulus is prolonged. The importance of vasodilatory mechanisms other than nitric oxide to clinical events remains largely unknown (see “Relative Contributions of Nitric Oxide, Prostacyclin & EDHF”, p.26).

Clinical implications. The use of different methodologies to evoke reactive hyperemia makes it difficult to compare the results between studies – at least mechanistically. However, FMD, independent of the modality used to evoke reactive hyperemia, may be an important independent barometer for risk of cardiovascular complications. Even though proximal occlusion may be less nitric oxide dependent than distal occlusion (64), FMD with proximal occlusion was actually more predictive of cardiovascular disease than FMD with distal occlusion (231). Further research is needed to determine whether FMD is a valid marker of cardiovascular disease risk independent of the modality used to induce FMD.
Importance of the Blood Flow Profile to Health

The aforementioned studies exposed cell cultures to blood flow profiles based on simplified mathematical models which assume parabolic flow and non-animated tubes. Cell culture studies offer the capacity to isolate singular components of the blood flow profile. However, the use of simplified flow profiles limits the capacity to translate these findings to the in vivo human haemodynamic environment. As already discussed, endothelial cell function is dependent not only on shear stress per se, but also upon flow pulsatility, velocity acceleration, flow direction, flow frequency, and flow turbulence. The exact interactions of these factors will be dependent on the make-up of the arterial wall itself – which differs markedly with anatomical location, age, and activity status.

Exercise

Despite the complications associated with understanding which flow profile parameters improve cultured endothelial cell functions, a number of in vivo human studies have indicated the potential clinical benefits of modulating flow pulsatility and velocity acceleration. Indeed, the noted atherosclerotic protective effects of physical activity may be induced by increased flow pulsatility and velocity acceleration. During physical activity, venous return, heart rates, and myocardial contractility are elevated. At low levels of muscular work, the increase in cardiac output is primarily derived from the increase in stroke volume. The increase in left ventricular preload augments the upstroke slopes of arterial pulsatile flow (161). In a numerical model, exercise changed local wall shear from an oscillating flow pattern to a predominantly pulsatile laminar flow pattern (175).
Exercise undoubtedly improves endothelial function. However, it is difficult to separate the relative importance of exercise-induced increases in blood flow and the indirect effects associated with reduced cardiovascular disease risk factors, e.g. blood lipids, circulating factors such as oxidants, blood pressure, blood glucose, and body fat. Nonetheless, a recent study did find that FMD improves after short-term exercise (8 weeks) without moderation of said factors (86). Furthermore, in a recent exercise therapy study, we found that endothelial function of an untrained arterial segment improved in patients with spinal cord injury (216). In that study, we resistance trained the thigh (the quadriceps femoris) using neuromuscular electrical stimulation. Measurements were made in the lower leg (the posterior tibial artery) down stream from the trained area. The posterior tibial artery would have indirectly experienced increases in blood flow since it is fed by the artery feeding the quadriceps femoris (the femoral artery) (195). The endothelial cells of the posterior tibial artery would, therefore, have been repeatedly exposed to increases in shear stress.

*External Counter-Pulsation Therapy*

External counter-pulsation is used to non-invasively increase cardiac output and flow pulsatility in vivo in humans (28, 146, 208, 237). During the therapy the patient lies supine while cuffs on the lower extremities (calfs, lower thighs and upper thighs) sequentially inflate at the beginning of diastole and deflate at the beginning of systole. Numerical simulation suggests that external counter-pulsation therapy results in rapid peripheral increases in shear stress during systole (i.e. velocity acceleration) followed by augmented forward flow during diastole (170). This therapy has been shown to result in
improved endothelial function (28, 29, 208), micro-vessel angiogenesis (245), and increased myocardial perfusion (221).

**Whole Body Acceleration Therapy**

Whole body acceleration is another form of therapy used to increase pulsatile blood flow. During therapy the subject lies supine on a motion platform which is driven repeatedly head to foot in a sinusoidal manner. Whole-body, periodic acceleration adds pulses to the circulation because fluid shifts occur within the body as the motion platform accelerates and decelerates (3). Heart rate, sympathetic activity, and cortisol are not affected, though blood pressure is (2). This therapy has been shown to: improve non-invasive markers of endothelial function (200), decrease the inhibitory effects of NOS inhibition (L-NAME) on FMD (4), and increase the release of endothelium-derived vasodilators (nitric oxide and prostacyclin) and tissue plasminogen activator antigen (2, 4, 199).

Both external counter-pulsation therapy and whole-body acceleration theoretically, and on visual inspection of pulse/velocity waveforms, increase velocity acceleration. However, this parameter has not been directly measured and related to markers of endothelial function. Furthermore, both therapies dramatically alter diastolic flow and arterial pressure.

**Summary**

- Exercise has been shown to improve endothelial function.
- Improvements in endothelial function may be contributable to exercise-induced increases in pulsatile shear stress.
• Counter-pulsation and whole body acceleration therapies have also been shown to improve endothelial function. These therapies increases pulsatile flow without increases metabolic rate.

Assessments of Endothelial Function

Endothelium-dependent agonists such as acetylcholine can be used to induce an endothelial response (76), but such practice is invasive and often unpractical, especially for use within clinical settings. Alternatively, a method which is non-invasive entails increasing shear stress by increasing blood flow demand downstream from the site of vessel imaging (Figure 2.16). This is the most common approach used in FMD studies. Typically a pneumatic tourniquet will be placed around the forearm just below the elbow and inflated to a super-systolic blood pressure for 5 min. When the occlusion is released the increased blood flow demand downstream (reactive hyperemia) will increase blood flow through the artery upstream, resulting in vasodilation of the artery by increasing shear stress. FMD is typically expressed as the percentage increase in the artery diameter from baseline to the peak vasoactive (diameter) response following reactive hyperemia.

Improving the Reliability of the Flow-Mediated Dilation Test

The within-subject variability of FMD has been reported to be as low as ~50% (63). This explains why a recent meta-analysis performed by Witte et al. (242) found that FMD was only related to low and not medium or high cardiovascular risk. Within any given study, FMD tests can consistently demonstrate a smaller degree of dilatation in atherosclerotic/risk factor patients versus controls. Subjects with only one cardiovascular risk factor report FMD values of ~7% (1). For a typical brachial artery of ~4 mm, this
translates to a 0.28 mm change in diameter. The pixel resolution of a typical ultrasound unit is ~0.04 * 0.04 mm. Measurements of 0.28 mm are within the standard error of measurements under stable resting conditions. However, maximal diameters in response to reactive hyperemia are short lived and therefore, hard to capture. Aside from standardizing measurement protocols, a number of suggestions can be made to improve measurement reproducibility.

Figure 2.16. Shear rate and diameter responses to 4 minutes ischemia. The horizontal line represents resting diameter. Flow-mediated dilation (FMD) is the peak percentage increase in diameter above rest.

Automated diameter measurements. Studies using automated diameter measurement have reported intersession coefficients of variation of ~14% (96, 243). A description of diameter measurements made in our lab is given in a following section.

Multiple measurements. Multiple shear rate/diameter measurements will make it more likely to achieve a non-biased estimate of FMD. Laws governing regression to the
mean suggest that a “true” response will be more likely reflected by multiple measurements (209). Capturing shear rate/stress – diameter slopes also decreases the likelihood of making erroneous conclusions. For instance, we recently found (to be submitted) that acute cigarette smoking attenuates the ratio of diameter: shear rate following 5 minutes ischemia. However, this ratio was consistently attenuated across a range of shear rates. The attenuation of FMD was overestimated when using single measurements.

*Flow-mediated dilation test calculations.* FMD can be calculated as: 1) post-only score, 2) change score, 3) fraction, or 4) co-varied for resting diameter. FMD is commonly presented as a fraction (the percentage change in diameter) whereas using resting diameter as a covariate is more likely to adjust for bias due to resting values (227, 228, 230). A simulation study found the greatest statistical power for the ANCOVA approach out of the four methods listed above, with fraction scores resulting in the lowest power (230).

*Ultrasound measures of cardiovascular health*

Arndt first applied ultrasound in 1968 to measure changes in the diameter of the carotid artery. Since then, the advancement of ultrasound technology has had a profound impact on the capacity of researchers and clinicians alike to non-invasively assess endothelial function and health. Most commercial ultrasound machines now provide duplex Doppler functionality; that is they can simultaneously image and measure blood velocity in conduit arteries in real-time (Figure 2.17). Duplex Doppler functionality offers immense potential for tracking vascular mechanical and functional changes related to atherosclerosis.
Arterial Diameter Measurements

Conventionally, two-dimensional brightness mode (B-mode) is employed to visualize in real-time the ultrasound echo amplitude distribution in a tomographic plane. Since the arteries of interest, except for the aorta, are within a depth range of 30 mm, a high carrier frequency (typically 7–13 MHz) can be used, resulting in detailed images of arteries, in both longitudinal and cross-sectional views (99). Reflections will only have prominent amplitude in the image if they originate from acoustic interfaces with a substantial change in acoustic impedance and are oriented perpendicular to the ultrasound beam direction. Therefore, in a cross-sectional view the lateral segments of the artery wall are blurred and with a relatively lower amplitude than the anterior and posterior lumen-wall transitions. In the longitudinal view, both walls will show up distinctly over a certain range, provided that the arterial segment considered is straight and without
branches. The transition of the inner layer of the wall, the intima to the lumen, induces a weak signal while the outer layer, the adventitia, results in reflections with high amplitude. The layer in between, the media, has a relatively low reflectivity and appears as a hypo-echoic band in images obtained with ultrasound systems with sufficient resolution.

Due to the advent of advanced image analysis programs, a number of laboratories are now able to make semi-automated measurements of diameter. In our lab we are able to make thirty diameter measurements per second. A video capture device (ADS Technologies) is used to make MPEG2 recordings at a rate of 30 frames per second. These video files are broken down, converted into JPEG images, and subsequently used to make 30 diameter measurements per second. JPEG images provide comparable accuracy for ultrasound image measurements compared to the DICOM (Digital Image and Communications in Medicine) standard (91). The images are analyzed offline using semi-automated edge-detection software (Figure 2.18) custom written to interface with National Instruments Lab view software (196, 215). Custom-written Excel Visual Basic Code is used to fit peaks and troughs to diameter waveforms in order to calculate diastolic, systolic, and mean diameters (Figure 2.19). Mean diameters are used for analysis.

To ensure image focus is maintained and that diameter waveforms are stable, the ultrasound probe needs to be fixed in place using a specialized probe holding device. The stability of diameter waveforms is also affected by rhythmic breathing patterns. To ensure optimal quality of diameter waveforms, the subject should hold their breath during
movie capture. In our laboratory between-day coefficients of variation for diameter measurements are 2.4-2.7% (214).

Figure 2.18. *Semi-automated diameter analysis*. The histogram (B) corresponds with the B-mode image (A) pixel brightness. The peaks (stars) correspond with the walls. Distance between brightest horizontal segments is recorded.

Figure 2.19. *Diameter waveforms from three cardiac cycles*. Green markers represent diastole, yellow represent systole.
Blood Velocity Measurements

Doppler ultrasound technology has been widely utilized to non-invasively measure blood velocity through conduit arteries in humans. Ultrasound assessments of blood velocity have been favorably compared to magnetic resonance imaging (159). With ultrasound blood velocity is calculated by measuring the Doppler effect, which results from a change in the frequency of a wave due to the motion of the wave source or receiver, or in the case of a reflected wave, motion of the reflector. The major reflector, in mammals is the red blood cell. The Doppler shift is dependent on the insonating frequency, the velocity of moving blood, and the angle between the sound beam and direction of moving blood, as expressed in the Doppler equation:

\[
Df = \frac{2 f v \cos q}{c},
\]

where: \(Df\) is the Doppler shift frequency (the difference between transmitted and received frequencies), \(f\) is the transmitted frequency, \(v\) is the blood velocity, \(c\) is the speed of sound, and \(q\) is the angle between the sound beam and the direction of moving blood. The equation can be rearranged to solve for blood velocity, and this is the value calculated by the ultrasound machine:

\[
V = \frac{Df c}{2 f \cos q}
\]

Since red blood cells travel at different speeds, even for a small measuring volume, there will be a range of blood velocities for a given time. Per cardiac cycle
Doppler ultrasound systems measure minimum, maximum, and mean blood velocities. These velocities are also time averaged across the cycle. Typically, studies use the time averaged mean velocities, though time averaged maximum velocities may be more accurate and reproducible, even though they may lead to overestimations of blood flow by ~40% (167).

Most commercial ultrasound units come equipped with software to automatically calculate blood velocities. The major limitation with these automated measurements is that they are calculated with each heart beat. In our laboratory, we rely on the automated measurements made by the ultrasound unit. These velocity measurements are recorded using optical character reading software. In our laboratory, between-day coefficients of variation for peak velocity measurements are 14.6% (214).

*Shear Stress Measurements*

Shear stress can be calculated as the product of shear rate and blood viscosity. Shear rate is typically estimated on the basis of Poiseuille’s law, using the equation:

\[
\text{Shear rate} = \frac{8v}{d}
\]

where \(v\) is the mean flow velocity of blood, and \(d\) is the mean internal arterial diameter.

In our laboratory, time-averaged maximum blood velocities are used to calculate shear rate. Time-averaged maximum velocity is the average of the highest velocities throughout the cardiac cycle. While time averaged maximum velocity overestimates blood flow by approximately 40% (based on time average mean velocity measurements.
made at the same time), it is the most robust velocity measurement (167). To estimate shear stress from shear rate, invasive measures of blood viscosity are required. However, for a given population, viscosity changes little. Shear stress assessments do not seem to result in conclusions different from shear rate assessments alone. Shear rate can therefore be used as an adequate surrogate measure (25, 81, 110, 180).

To estimate shear rate, blood velocities and diameter, measurements need to be synchronized. In our laboratory, the rate at which we measure shear rate is limited to the rate at which the ultrasound unit calculates blood velocities, i.e., with every heart beat.

**Non-Invasive Methods to Increase Blood Flow**

In order to investigate the relationship between shear stress and FMD, blood flow through the region of interest must be *indirectly* manipulated. That is, the manipulation itself must not affect the region of interest. If the brachial artery is the region of interest, blood flow can be indirectly elevated using forearm ischemia, handgrip exercise, or forearm heating.

*Ischemia.* Ischemia is the standard approach for FMD. Typically, a pneumatic tourniquet will be placed around the forearm just below the elbow and inflated to a super-systolic blood pressure for 5 min. When the occlusion is released, the increased blood flow demand downstream (reactive hyperemia) will increase blood flow through the brachial artery. This is a simple and validated approach. The major disadvantage lies in the transient and turbulent nature of the increased blood flow. Measurements of the flow profile are difficult under these conditions.

*Exercise.* Rhythmic handgrip exercise can also be used to increase blood flow. Handgrip exercise increases metabolic demand of the forearm. The role of the
endothelium in exercise-induced vasodilatation is not clear. A possible limitation is the potential for recruitment of the bicep muscle, thereby directly activating the region of interest. The exercise intensity has to be of a low enough intensity to prevent synergistic muscle activity. Electromyography can be used to ascertain that the bicep remains inactivated.

Heating. Local forearm heating also results in increased blood flow demand to the forearm. Local warming of the skin induces localized dilation that is graded with skin temperature with the maximal dilation and blood velocity response occurring at 42°C (112, 223). Pilot work in our lab corroborates that heating the forearm to 42°C elicits a maximal increase in blood flow to the forearm. Warming of the skin is thought to increase blood flow locally without significant systemic autonomic influence (112, 115, 207, 223). The mechanism responsible for this response is not fully understood, but endothelial nitric oxide production is thought to play a central role (114, 115, 207). There is evidence to suggest that that this response may be produced through a neurogenic reflex with nitric oxide serving a permissive role to some unknown neurotransmitter (51).

Under controlled conditions, gradually increasing skin temperature can induce successive, sustained, and reproducible increases in local blood flow(111, 180, 203, 223). To ensure that the brachial artery is not directly heated, the forearm has to be encased within an airtight container. The skin temperature of the bicep should be continuously monitored.

Can FMD be induced by non-shear stress dependent pathways when flow is indirectly increased? Given that the endothelium is capable of conducting a vasodilatory signal along the vascular tree (19), there is concern that heating and forearm exercise may
induce vasodilation independent of shear stress. However, studies have shown that hand warming has no effect on brachial artery diameter when flow is not allowed to rise (110, 154, 180). Furthermore, pharmacological blockade of the autonomic nervous system has no effect on radial artery FMD in response to hand warming (154), consistent with animal studies showing that FMD is preserved after surgical or pharmacological denervation (97, 130).

*Measuring velocity acceleration*

Velocity acceleration can be measured using conventional image analysis software such as Image J (Open Source). The following calculations can be used to estimate velocity acceleration:

$$\text{Velocity Acceleration cm/s}^2 = \frac{\text{SV} - \text{ED}}{\text{acceleration time}}$$

or

$$\text{Velocity Acceleration cm/s}^2 = \left(\frac{\text{SV} - \text{ED}}{\text{acceleration time}}\right) \times \left(\frac{HR}{60}\right)$$

where SV = systolic velocity, ED = end diastolic velocity. The second calculation normalizes velocity acceleration to heart rate. Figure 2.20. shows how SV and ED coordinates are identified.

There does not appear to be a consensus as to how to quantify velocity acceleration. Crutchfield *et al.* (52) simply measured velocity acceleration from the onset of systole to the point of peak systole. Chung *et al.* (46) fitted a triangle to the velocity waveform, and used the foot to peak of the triangle to represent acceleration. However, both of the aforementioned methods entail identification of the peak. The peak is often
hard to identify. A more robust method was utilized by Mitchell et al. (148), who measured from the onset of systole to the point at which velocity reaches 95% of peak.

There is a need to normalize velocity acceleration to heart rate. Heart rate dependence has been demonstrated for the shape of the blood flow profile. Velocity acceleration, peak flow rate, stroke volume, and cardiac output all increased in proportion to heart rate (45, 46, 59, 247). The duration of diastole is inversely related to heart rate (45).

Figure 2.20 *Velocity acceleration calculation*. 1 = end diastolic velocity, 2 = 95% of peak systolic velocity, 3 = peak systolic velocity, 4 = minimum diastolic velocity.

In our laboratory, measurements are made off-line on the waves with the strongest overall signal (determined by visual inspection of the signal intensity) and with maximum systolic velocities. This technique minimizes error related to insonation angle.
**Manipulating Velocity Acceleration**

Velocity acceleration reflects the force of blood flow, which is met with an equal opposing force created by the downstream resistance. The force of the blood flow is largely governed by myocardial contractility (233). Flow resistance modulates velocity acceleration by reflecting pressure waves (40). Resistance to flow is principally supplied by distal arterial beds (34, 89, 90). Flow in high-resistance conduit arteries is characterized by rapid velocity acceleration, decreased systolic duration, and flow reversal during early diastole (90). Finally, flow resistance is also modulated by arterial compliance (34). More compliant vessels are able to store a greater portion of kinetic energy from each systolic pulse (89). As compliance increases, velocity acceleration decreases (40).

Myocardial contractility can be increased with exercise (108) or positive inotropic agents (43), or decreased with negative inotropic agents or beta blockers (43). However, altering myocardial contractility would result in unwanted systemic effects. An alternative approach is to non-invasively alter resistance by inflating a tourniquet around the mid-forearm to a low pressure (40 mmHg).

**Blood Pressure Measurements**

Blood pressure can essentially be defined as ‘the force of blood against the wall of the arteries’. The surge of blood that occurs at each contraction is transmitted through the elastic walls of the entire arterial system where it can be detected as the pulse. We can therefore use measurements of blood pressure as an indirect index of circumferential strain.
There are a number of non-invasive techniques for measuring blood pressure. In our laboratory, we can make blood pressure measurements using standard auscultatory methods or a semi-automated blood device (Accutor 3, Datascope). We can also measure blood pressure using a finger photo-plethysmographic device (Finapress 2300, Ohmeda), or using radial artery tonometry (Colin 7000, Colin). The later two methods allow us to make continuous blood pressure measurements. However, these methods are not suitable for making measurements on the arm that is being insonicated during ultrasound studies.

Circumferential strain has also been calculated using the energy conservation law to estimate potential energy (ΔP) (150). Blood pressure is an estimate of potential energy, and is actually only one form of energy available for moving blood. Total fluid energy (E) per unit volume of blood equals the sum of the potential energy, gravitational potential energy, plus kinetic energy:

\[
\text{TFE} = P + G + K
\]

where \( P \) is the potential energy, \( G \) is the gravitational energy, and \( K \) is the kinetic energy.

\[
\text{TFE} = P + \rho gh + \frac{1}{2} \rho v^2
\]

where \( P \) is the potential energy, \( \rho \) the blood density, \( g \) the earth’s gravity acceleration constant, \( h \) the height of the fluid above or below a reference level, and \( v \) the
flow velocity. If all parts of the fluid system are at the same level (i.e. when supine), gravitational potential energy can be ignored. The equation can thus be written:

\[ TFE = P + \frac{1}{2} \rho v^2 \]

Total fluid energy (TFE) is constant (energy conservation law). When the left ventricle contracts, all the energy imparted to the blood does not appear immediately as kinetic energy. During systole when the vessel diameter widens, potential energy is stored in the artery wall. At diastole, the recoil of the elastic tissue returns the energy stored in the arterial wall to the blood, so the potential energy decreases. The potential energy in arteries is therefore reciprocally related to variations in blood flow velocities through the cardiac cycle. Potential energy, therefore, can be estimated using the formula:

\[ \Delta P = \frac{1}{2} \rho (V_1^2 - V_2^2) \]

where \( V_1 \) and \( V_2 \) are the peak systolic velocity and end diastolic velocity, respectively. Blood density (\( \rho \)) is assumed to be 1060 kg/m\(^3\). The natural unit of expression is dyn/cm\(^2\).

The above equation ignores blood viscosity which may affect readings, but accurate correlations between Doppler and catheterization methods have been shown at high altitude where viscosity is increased (6). A distinct advantage to this methodology lies in the capacity to directly estimate pressure in the specific region of interest. Most
studies estimating circumferential strain measure blood pressure in the brachial artery of the opposite arm.

Estimates of static pressure have previously been used to calculate a variation of the pressure – strain modules (Ep) (150):

\[
E_p = \frac{\Delta P}{\Delta D/D_d}
\]

where \(\Delta D/D_d\) is arterial strain, \(\Delta D\) is the change in diameter over the cardiac cycle and \(D_d\) is the diastolic diameter. This method was successfully used to discriminate the degree of stenosis in the carotid artery of 128 men (66 ± 11 yr). Discriminate analysis correctly classified the degree of stenosis (normal, mild or severe) for 94% of the cases. Ep was also calculated using the conventional approach with brachial artery pulse pressure. Only 40% of cases were correctly classified using the later approach.
CHAPTER III

VELOCITY ACCELERATION AS A DETERMINANT OF

FLOW-MEDIATED DILATION¹

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ABSTRACT

Shear stress is the established stimulus for flow-mediated dilation (FMD). In vivo, shear stress is typically estimated using mean blood velocity. However, mean blood velocity may not adequately characterize the shear stimulus. Pulsatile flow results in large shear rate gradients (velocity acceleration) at the onset of flow. The purpose of this study was to determine the importance of velocity acceleration to FMD. We define FMD as the shear rate-diameter relationship. We hypothesized that increasing velocity acceleration does augment FMD. Fourteen physically active, young (26±5 years), male subjects were tested. FMD was assessed using progressive forearm heating and handgrip exercise to elicit steady-state increases in shear rate. FMD was measured prior to and following induced increases in velocity acceleration. Velocity acceleration was increased by inflating a tourniquet around the forearm to 40 mmHg. Hierarchical linear modeling (HLM) was used to estimate change in diameter with repeated measures of shear stress nested within each subject. Velocity acceleration was used as a covariate. Averaged across conditions the 40 mmHg cuff resulted in a 14% increase in velocity acceleration (P = 0.001), and 17% decrease in mean velocity (P = 0.006). The shear rate-diameter slope was 3.39 for the control condition and 3.02 with increased velocity acceleration, a decrease of 11% (P = 0.015). This finding suggests velocity acceleration attenuates FMD in physically active, young males.

INDEX WORDS: Flow-mediated dilatation, velocity acceleration, flow pulsatility, shear stress, shear stress.
INTRODUCTION

Disruption of the endothelium lining of conduit arteries is thought to occur early in the pathogenesis of cardiovascular disease (38). The flow-mediated dilation (FMD) test is widely used to non-invasively assess in vivo endothelial function (10). Reduced FMD reflects an impaired capacity of the endothelium to maintain vascular tone by releasing vasodilators, including nitric oxide (31), and is used as a barometer of endothelial health. Reduced FMD is an early marker of atherosclerosis (10) and has been noted for its predictive capacity for future cardiovascular complications (19, 49). Despite its potential, the validity of FMD test has been questioned due to the lack of normalization to the primary stimulus (29, 34).

Despite the term flow-mediated dilatation, shear stress is the established stimulus for FMD (27). Shear stress estimates are typically based on Poiseuille’s law and mean blood velocity is assumed to be the primary parameter governing the shear stimulus. However, pulsatile flow, present throughout the arterial tree, results in endothelial cells being exposed to two distinct shear stimuli during the cardiac cycle: a large rate of change (velocity acceleration) in shear at the onset of flow, followed by relatively steady shear. In vitro studies suggest that these two distinct fluid stimuli regulate short- and long-term endothelial function via independent biomechanical pathways (16, 18, 28). Velocity acceleration may be an important independent variable governing the shear stimulus.

The purpose of our study was to determine the importance of velocity acceleration to FMD. The FMD phenomena was assessed using progressive forearm heating and handgrip exercise to elicit steady-state increases in shear rate. For this study FMD is
defined as the shear rate-diameter slope. FMD was measured prior to and following induced increases in velocity acceleration. We hypothesized that velocity acceleration independently predict change in diameter.

METHODS

Subjects and Study Design

Fifteen healthy, physically active, young male subjects were recruited. None of the subjects reported cardiovascular disease complications. The study was approved by the University of Georgia Institutional Review Board. Informed consent was obtained from the subjects after they were given a detailed description of the procedures.

The test sessions were performed between the hours of 7 am and 10 am to reduce circadian variation. All subjects were asked to report to the laboratory in the fasted condition and having refrained from exercise for 48 hours prior to testing. Subjects were also asked not to consume caffeine or supplements with known vascular actions. Subjects were excluded from the study if they were currently prescribed medications with known vascular actions.

Protocol

Each subject was tested on two days (control and acceleration conditions) separated by less than 7 days. The five stages of the control condition are shown in Figure 3.1. Testing commenced following at least 10 minutes of quiet supine rest. During the acceleration condition, resting measurements were made following quiet rest. Velocity acceleration was then increased by inflating a pneumatic tourniquet placed around the mid-forearm to a pressure of 40 mmHg. Inflation of the tourniquet increased velocity acceleration through the creation of pulse wave reflections (9). The five stages of
testing (Figure 3.1) commenced after 5 minutes of tourniquet inflation. The tourniquet remained inflated for the duration of the experiment. Left arm brachial artery diameters and blood velocities were recorded during the last 30 seconds of each stage. There was concern that inflation of the tourniquet would alter circumferential stress. Therefore, two estimates of circumferential stress were also measured at the end of each stage.

*Indirect Local Heating*

The subject’s forearm was placed into a specially constructed air-tight container. The base of the container was lined with a digital moist heating pad and an inlet was used to circulate warm air. Warming of the skin is thought to increase blood flow locally without significant systemic autonomic influence (25, 47). The forearm was heated to 40°C for 10 minutes and then to 42°C for 5 minutes. Forearm and bicep skin temperatures were monitored using fast response temperature probes (TSD202B, Biopac Systems, Inc., Goleta, California).

*Forearm Exercise*

Two stages of isometric rhythmic handgrip exercise were performed using a handgrip ergometer. For each stage the subject was asked to squeeze to 10% of their maximal voluntary contraction (MVC). Each stage lasted 4 minutes. For stage one the subject contracted once every 3 seconds. For stage two the subject contracted twice every 3 seconds. Low intensity handgrip exercise of a small muscle group was chosen to minimize systemic autonomic responses. Subjects were encouraged to perform the exercise with minimal bicep activity. Bicep electrical activity was monitored using electromyography (EMG 100C, Biopac Systems, Inc., Goleta, California). Force output
was captured continuously (MP100, Biopac Systems, Inc., Goleta, California) and visual feedback was provided to the subject. Velcro straps were used to fasten the forearm.

**Ultrasound Diameter Measurements**

High-resolution B-mode ultrasound measurements were made using a GE 400CL duplex color Doppler unit (GE Medical, Milwaukee, Wisconsin) equipped with a 7-13 MHz linear array transducer (LA39). Ultrasound global (acoustic output, gain, dynamic range, gamma, and rejection) and probe-dependant (zoom factor, edge enhancement, frame averaging, and target frame rate) settings were standardized. Care was taken to ensure that the vessel clearly extended across the entire [un-zoomed] imaging plane to minimize the likelihood of skewing the vessel walls. The image was adjusted until the vessel walls appeared thickest. The image was then zoomed to focus on the center of the vessel. The image was comprised of 400*400 pixels, an area of 16*16 mm with a pixel resolution of 0.04*0.04 mm. Image focus was maintained using a specialized probe holding device. The probe holding device allowed fine adjustments to probe positioning and was used to elevate the probe from the skin to avoid pressure being applied to the artery.

**Diameter analysis**

Moving Picture Experts Group-2 (MPEG-2) recordings were captured using a Dell Laptop PC equipped with a with video capture device (ADS technologies, Cerritos, California). Video files collected at 30 frames/second were converted into Joint Photographic Experts Group (JEPG) images and subsequently used to make 30 diameter measurements/second. JPEG images provide comparable accuracy for ultrasound image measurements compared to the Digital Image and Communications in Medicine
DICOM) standard (22). Two 10 second movies were taken for each stage of testing. Subjects were asked to hold their breath during movie capture. Images were measured offline using semi-automated edge-detection software custom written to interface with the LabVIEW data acquisition platform (version 8.1, National Instruments, Austin, Texas) (39, 46). Custom written Excel Visual Basic code was used to fit peaks and troughs to diameter waveforms in order to calculate diastolic, systolic, and mean diameters. Mean diameters were used for analysis. The within-session SEM for the described set-up is 0.046 mm. Between-day coefficients of variation are 2.4-2.7% (45).

**Blood Velocities**

Sonication angle was kept constant between 45-65° and the sample volume included most of the vessel. Blood velocities were calculated using the advanced vascular package supplied with the GE 400CL ultrasound machine. A custom written Optical Character Reading (OCR) package written in LabVIEW was used to capture blood velocities for each cardiac cycle. When more than two values were recorded for a given second, the average was computed. Obvious outliers were removed and missing values were replaced using linear interpolation. In our laboratory, between-day coefficients of variation for peak velocity measurements are 14.6% (45).

**Shear Rate**

The 30 diameters measurements/second were aggregated to 1/second and synchronized with blood velocities. Shear rates were calculated as the product of:

\[
\text{Shear Rate (s}^{-1} \text{)} = \frac{8 \times \text{mean blood velocity}}{\text{diameter}}
\]
Time averaged maximum blood velocities were used to calculate shear rate. Time averaged maximum velocity is the average of the highest velocities throughout the cardiac cycle. Our ultrasound machine more reliably calculates these velocities when compared to time averaged mean blood velocities (33).

**Velocity Acceleration.**

Blood velocity acceleration was estimated using ImageJ image processing and analysis software (36):

\[
\text{Velocity Acceleration (cm/s}^2) = (((SV - ED) / \text{acceleration time}) \times (HR / 60))
\]

where \(SV\) = systolic velocity, \(ED\) = end diastolic velocity, and \(HR\) = heart rate. Figure 3.2 shows how systole velocity and end diastolic coordinates were identified. Velocity acceleration was normalized to heart rate since velocity acceleration is modulated by heart rate (12).

**Circumferential Stress**

Circumferential stress was indirectly estimated using conventional pulse pressure measurements. Since pulse pressure measurements could not be directly taken on the Ultrasound imaged arm, circumferential stress was also estimated using the energy conversion law (30).

**Pulse Pressure.** Blood pressures were continuously measured on the right (non-imaged) arm using a finger photo-plethysmographic device (Finapress 2300, Ohmeda, Englewood, Colorado) interfaced to a Biopac data acquisition system (MP100, Biopac Systems, Inc., Goleta, California). To ensure the Finapress was correctly calibrated, the
systolic and diastolic blood pressure values were checked against recordings from a semi-automated blood pressure device (Datascope Accutor 3, Montvale, New Jersey). If systolic and diastolic measurements did not agree within 5 mmHg, the finger cuff was adjusted until the two devices agreed (42).

Potential Energy. Blood pressure is an estimate of potential energy and represents just one source of energy in a given arterial segment. Total fluid energy per unit volume of blood equals the sum of the potential energy, gravitational potential energy, plus kinetic energy. If all parts of the fluid system are at the same level (i.e. when supine), gravitational potential energy can be ignored. Since total fluid energy is constant (energy conservation law) potential energy and kinetic energy are reciprocally related (30). Potential energy, or static pressure, can be calculated using the formula:

$$\Delta P_s = \frac{1}{2} \rho \left( V_1^2 - V_2^2 \right)$$

where $V_1$ and $V_2$ are the peak systolic velocity and end diastolic velocity, respectively. Blood density ($\rho$) was assumed to be 1060 kg/m$^3$.

Statistics

Descriptive data for each stage of testing are expressed as means (+SD). Between-condition resting measurements were compared using 2-tailed dependant student’s $t$ tests. The primary study outcomes were analyzed using hierarchical linear modeling (HLM) with the HLM6 (Scientific Software International, Inc., Lincolnwood, IL) statistical package. A key application of HLM relates to the capacity to account for correlated measures by recognizing the nested data structure (37), i.e. repeated measures nested within each subject. This approach models different patterns of growth trajectories by
allowing for the intercepts (initial diameter) and slopes (shear rate-diameter) to randomly vary (37). Briefly, HLM was used to compare the shear rate-diameter relationship for the control vs. acceleration conditions. The final within-subject (level 1) and between-subject (level-2) models were specified:

**Level-1 Model**

\[ Y_{si} = \pi_{0i} + \pi_{1i} *(SR_{si}) + \pi_{2i} *(SR *\text{Cond}_{si}) + \pi_{3i} *(VA_{si}) + e_{si} \]

where \( \pi_{0i} \) (intercept) represents diameter for person i when \( SR_{si} = 0 \), \( SR \) = shear rate, and \( \pi_{li} \) (slope) represents change in diameter per 1 unit shear rate, i.e. FMD, \( \text{Cond} \) represents a dummy coded variable to identify control (0) and velocity acceleration (1) conditions, and \( VA \) represents stage-centered velocity acceleration. The intercept and slopes specified at level 1 become outcomes at level 2.

**Level-2 Model**

\[ \pi_{0i} = \beta_{00} + r_{0i} \]
\[ \pi_{1i} = \beta_{10} + r_{1i} \]
\[ \pi_{2i} = \beta_{20} \]
\[ \pi_{3i} = \beta_{30} \]

where \( r_{0i} \) and \( r_{1i} \) are the unique increments associated with individual i, indicating that the individual intercepts and slopes were allowed to randomly vary.
Level 2 outcomes were used for hypothesis testing. Two HLM models were estimated to test the hypothesis. Each of these models were initially specified as unconditional, i.e., using shear rate as the sole predictor to provide estimates of the shear rate-diameter slopes (FMD). Additional predictor variables were subsequently specified within sub-models. Statistical significance was defined as $p < 0.05$.

*Model 1.* The initial sub-model specified shear rate as a single predictor variable to provide a global unconditional estimate of the shear rate-diameter slope (FMD). The unconditional model was also used to estimate measurement reliability. The estimated reliability is defined as the ratio between the level-2 variance component and the sum of the level-2 and level-1 components, with the latter divided by the number of observations (37). Poor reliability (below 0.10) would render the data incapable of identifying relationships between variables for the given population sample size (37). The relationship between the slope (FMD) and the intercept was calculated as the intra-class correlation coefficient between intercept and slope random variance components.

The subsequent sub-model specified velocity acceleration to determine whether this variable explained a significant portion of variation in addition to shear rate. The study hypothesis was accepted if velocity acceleration significantly predicted change in diameter. To account for possible changes in circumferential stress, our two estimates (pulse pressure and potential energy) were univariately regressed against mean diameter. The percentage within-subject variance explained by each explanatory variable was calculated. For the final model, the circumferential stress parameter which explained the most univariate within-subject variation was specified in addition to shear rate and velocity acceleration.
Model 2. The initial model specified shear rate and the interaction term $SR*Cond$. The interaction term was used to determine whether the shear rate-diameter relationship (FMD) significantly differed by condition. Velocity acceleration and circumferential stress (potential energy) estimates were centered by stage and specified as covariates to determine whether these variables can explain differences in FMD between conditions. The study hypothesis was accepted if the shear rate-diameter slopes were significantly different between conditions. The hypothesis was verified if the difference in FMD between conditions became non-significant after specifying velocity acceleration.

Effects of condition on study variables. Each study variable was specified as an HLM outcome. A dummy coded condition variable was the single additional parameter specified.

RESULTS

Analysis was conducted on 14 of the 15 subjects. One subject was omitted due to poor B-mode ultrasound image quality. For the remaining fourteen subjects the average (±SD) age was 26±5 years, height 177±7 cm, and weight 78±9 kg. Subjects exercised an average of 6±3 times a week for 67±29 minutes/session. Average systolic, diastolic and mean blood pressures were 120±9 mmHg, 70±6 mmHg, and 89±7 mmHg, respectively. Blood pressure did not significantly differ between conditions.

Effects of condition on study variables

Condition did not significantly alter resting diameters (4.14±0.44 mm vs. 4.17±0.43 mm, $P = 0.113$) shear rates (351±180 s$^{-1}$ vs. 330±219 s$^{-1}$, $P = 0.356$), pulse pressures (51.8±6.3 mmHg vs. 50.6±7.4 mmHg, $P = 0.277$), or hear rates (59.7±8.9 bpm vs. 59.2±9.2 bpm, $P = 0.704$).
Figure 3.3 shows representative examples of velocity waveforms during rest and exercise for each condition. Figure 3.4 shows velocity acceleration plotted against shear rate. Velocity acceleration, relative to shear rate, was increased by 40% across all stages for the acceleration vs. control condition. Estimates of circumferential stress using pulse pressure (P = 0.900) and potential energy (P = 0.339) were not significantly affected by condition. Figure 3.5 shows the mean average change in diameters and shear rates across stages and between conditions. Across all stages, the acceleration condition attenuated diameters (P = 0.001) and increased velocity acceleration (P = 0.001). The acceleration condition also resulted in a small non-significant (P = 0.052) attenuation of shear rates.

**Model 1**

Figure 3.6 shows vessel diameter plotted against shear rate. Vessel diameters in particular demonstrate a large amount of variation. The majority of this variation can be explained by two factors. Firstly, resting diameters ranged 3.05-4.74 mm. Individuals with larger initial diameters would be expected to have greater absolute FMD responses. Secondly, the standard deviations are misleading. The calculation of standard deviations forces the continuous data to become categorized according to stage. The shear rate responses varied notably between subjects, this would influence the magnitude of FMD. This explains why, despite the variation, the reliabilities for the intercept and slope were 0.96 and 0.59, respectively. These results signify that despite differences in initial diameter, change in diameter can be adequately predicted by shear rate. These results also indicate that there was adequate signal in the data to specify additional predictor variables and detect the resultant effect.
Four HLM sub-models were specified. Table 3.1 shows the unconditional and final conditional sub-models. The initial model specified shear rate as the single predictor variable. The intra-class correlation between the intercept and slope was -0.45, indicating that subjects with larger initial diameters had lower FMD. The second sub-model specified velocity acceleration, which explained a significant portion of variation beyond shear rate ($\beta = -0.110 \times 10^{-4}$, $P = 0.035$). The third sub-model specified pulse pressure, which did not explain a significant portion of variation beyond shear rate and velocity acceleration. The final sub-model specified circumferential stress (potential energy), which did explain a significant ($\beta = 0.042$, $P = 0.013$) portion of variation. The addition of velocity acceleration and circumferential stress predictors improved the reliability of the slope (FMD) to 0.70. The purpose of this study was to explain within-subject (level-1) variance, i.e., predict FMD for each subject, not to explain differences between-subjects (level-2). We did not expect to decrease between-subject variance. However, it is worth noting that the between-subject random variance for intercept and slope parameters remained significant. The addition of between-subject subject predictors, e.g., resting diameter, age, weight etc., may have improved the capacity to predict FMD.

Model 2

Three HLM sub-models were specified. Table 3.2 shows the unconditional and final conditional models. The initial model specified shear rate and the shear rate * condition interaction term. The FMD slope was 11.0% slower ($P = 0.015$) for the acceleration vs. control condition. The second sub-model specified velocity acceleration centered by stage. This was to determine whether velocity acceleration explained the significant difference in FMD between conditions. After specifying velocity acceleration,
FMD was no longer significantly different between conditions ($\beta = -0.113 \times 10^{-4}$, $P = 0.619$). The final model specified circumferential stress (potential energy) centered by stage. FMD between conditions remained non-significant ($P = 0.564$).

**DISCUSSION**

This study estimated FMD phenomena using the relationship between shear rate and vessel diameter. A novel aspect of this study was the measurement and manipulation of velocity acceleration. Velocity acceleration, in addition to shear rate, was found to predict FMD. The major finding of this study was the attenuated FMD in response to induced increases in velocity acceleration. This finding suggests that mean blood velocity alone may not adequately define the shear stimulus.

Over the past two decades, cell culture studies have demonstrated that temporal shear gradients (induced by velocity acceleration) can influence the progression of atherosclerosis (3, 15), mechanotransduction (2, 24), calcium kinetics (4, 23), and vascular tone (16, 32). Notably, the release of nitric oxide and prostacyclin from cultured endothelial cells has been directly related to the rate of velocity acceleration (17, 18, 28, 32). Velocity acceleration has also been directly related to vasodilation of isolated cremaster arterioles (8). Shear stress and velocity acceleration have been shown to mechanotransduce signals, which regulate vascular tone, via different pathways (16, 18, 28). Steady-state shear stress-induced nitric oxide production is thought to be Ca$^{2+}$/G-protein-independent. In contrast, acceleration-induced nitric oxide production is thought to be Ca$^{2+}$/G-protein-dependant. Dusserre et al. (16) demonstrated that velocity acceleration activates platelet/endothelial cell adhesion molecule-1 (PECAM-1) (16).
which is complexed with eNOS at the cell-cell junction. The eNOS/PECAM-1 complex was activated by velocity acceleration but not by shear stress *per se*.

The inhibitory effects of velocity acceleration on FMD in the current study may be explained in a number of ways: 1) induced-velocity acceleration may have altered additional secondary flow phenomena, 2) endothelial cells have an optimal frequency at which they respond to secondary flow phenomena, and 3) induced-velocity acceleration altered circumferential stress. Firstly, the delicate balance between vasoconstrictor (e.g., endothelin-1) and vasodilator (e.g., nitric oxide and prostacyclins) factors released by the endothelium may have been negatively affected if induced-velocity acceleration increased flow turbulence or decreased the steady flow component during diastole (32). Oscillatory flow (flow devoid of a steady component) and turbulent flow have both been shown to inhibit the release of vasodilators and promote the release of vasoconstrictors (32). Turbulence has been estimated using the ratio between time average maximal velocity and time average mean velocity (6). We found no evidence that tourniquet inflation increased turbulence. However, on average diastolic velocity decreased by 32%, indicating a decrease in the steady flow component.

Secondly, the need for sensitive measurements required that we recruited an optimal population sample. Therefore, we recruited physically active, young males. The endothelial cells of our subjects were likely already being exposed to optimal haemodynamic conditions. In vitro, it has been demonstrated that different tissue beds exhibit different optimal flow frequencies for proliferation, eNOS activity, and prostacyclin secretion (1). Induced-velocity acceleration may have resulted in less than optimal haemodynamic conditions in our physically active, young subjects. It is plausible
that induced increases in velocity acceleration would not attenuate FMD in less healthy populations. Flow pulsatility has been manipulated in vivo in humans using external counter-pulsation- and periodic acceleration-therapy. These therapies, which increase pulsatile blood flow and velocity acceleration without altering metabolic demand, have improved non-invasive markers of endothelial function (41, 43). However, these therapies have not been performed on physically active, young subjects. There remains a need to address whether different populations respond differently to a given flow paradigm.

Lastly, while we did not find evidence for altered circumferential stress, the methodology used to induce velocity acceleration may have altered interactions between shear- and circumferential-stress. Velocity acceleration reflects the force of blood flow, which is met with an equal opposing force created by the resistance to flow. The force of the blood flow is largely governed by myocardial contractility (48). Flow resistance, supplied by distal arterial beds (7, 21) and vessel compliance (9, 20), modulates velocity acceleration by reflecting pressure waves (9). Flow in high-resistance conduit arteries is characterized by rapid velocity acceleration, decreased systolic duration, and flow reversal during early diastole (21). Altering myocardial contractility would have resulted in confounding systemic effects. We therefore, non-invasively altered flow resistance by inflating a tourniquet around the mid-forearm to a low pressure (40 mmHg). This may have resulted in an unwanted side-effect. The wave reflection as a result of tourniquet pressure may have caused a time lag to occur between shear- and circumferential-stress, know as a stress phase angle. Qui and Tarbell (35) found that the addition of circumferential stress in concert with oscillatory shear stress tends to enhance the
production of vasodilators. However, they also found that when circumferential stress and shear stress waveforms were asynchronous, vasodilator production was inhibited. Asynchronous circumferential and shear stress generates a complex, time-varying mechanical force pattern on the endothelial cell layer. Several studies have demonstrated that large stress phase angles are pro-athergoneic and inhibit flow-induced release of vasodilators (13, 14, 35).

**Clinical Implications**

Velocity acceleration may be decreased (26, 40) or increased (11, 42) by a number of disease states. Velocity acceleration also decreases with age (42) and increases with physical activity (5, 43). We found that FMD was attenuated by induced increases in velocity acceleration despite no alteration in endothelial function. This finding suggests that comparisons between disease groups may be misleading in the presence of differing secondary flow phenomena. Similarly, secondary flow phenomena may be altered during the course of interventional studies e.g., through enhanced myocardial contractility following exercise therapy.

**Limitations**

Human studies of FMD usually entail measuring change in diameter in response to transient reactive hyperemia. The current study induced sustained increases in shear rate. Sustained vs. transient shear rate was chosen for a number of reasons: 1) improved accuracy of sensitive measurements, 2) transient increases in shear rate are difficult to quantify, and 3) the accuracy of shear rate measurements during reactive hyperemia are limited by the presence of turbulent flow. However, both acute and chronic regulation of vascular tone is an important function performed by the endothelium. Further research is
needed to determine the importance of secondary flow phenomena to acute control of vascular tone.

Summary and Conclusions

We found that induced increases in velocity acceleration attenuated FMD phenomena. FMD was attenuated despite no alteration in endothelial function. Previous in vitro studies have shown that endothelial cell regulation is dependant not only on shear stress *per se*, but also upon shear stress gradients (velocity acceleration). However, these in vitro studies have reported velocity acceleration to have a positive effect on the release of endothelium-derived dilatory factors. Our subjects were physically active, young males. It remains to be determined whether velocity acceleration similarly affects less healthy populations. Attention to secondary flow phenomena may be particularly important when comparing groups with known secondary flow abnormalities.
REFERENCES


12. **Chung CS, and Kovacs SJ.** Consequences of increasing heart rate on deceleration time, the velocity-time integral, and E/A. *Am J Cardiol* 97: 130-136, 2006.


FIGURE LEGENDS

Figure 3.1. *Five protocol stages.* For the heating stages only the forearm was heated. For the exercise stages the forearm remained heated to 42ºC. Exercise was performed at 10% MVC. Total testing time was 24 minutes.

Figure 3.2. *Velocity acceleration calculation.* 1 = end diastolic velocity. 2 = 95% of peak systolic velocity. 3 = peak systolic velocity. 4 = minimum diastolic velocity.

Figure 3.3. *Representative blood velocity waveforms.* Wave forms are shown for the first (rest, A = control condition, C = acceleration condition 2) and last stages (exercise, B = control condition, D = acceleration condition 2). VA = velocity acceleration. Vm = time averaged mean velocity. Vs = peak systolic velocity. Vd = end diastolic velocity.

Figure 3.4. *Effects of condition on velocity acceleration* Control refers to control condition. Acceleration refers to increased velocity acceleration condition.
Figure 3.5. *Effects of condition on diameter (A) and shear rate (B) change.* **Control** refers to control condition. **Acceleration** refers to increased velocity acceleration condition. Representative standard deviations are shown.

Figure 3.6. *Effects of velocity acceleration on flow-mediated dilation.* **Control** refers to control condition. **Acceleration** refers to increased velocity acceleration condition. Representative standard deviations are shown.
Table 3.1. *HLM estimates for change in diameter with shear rate.*

<table>
<thead>
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<th>Uncond.</th>
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<th>Conditional</th>
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<td></td>
<td>Est.</td>
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<td>Fixed Effects</td>
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</tr>
<tr>
<td>Intercept $\beta_{00}$ $D_{\text{mean}}$</td>
<td>4.048</td>
<td>&lt;0.001</td>
<td>4.194</td>
<td>&lt;0.001</td>
</tr>
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<td>P1</td>
<td>$\beta_{10}$ $\text{SR}$</td>
<td>$3.377^{10^{-4}}$</td>
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<tr>
<td>P2</td>
<td>$\beta_{20}$ $\text{VA}$</td>
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<tr>
<td>P3</td>
<td>$\beta_{40}$ $\text{CS}$</td>
<td>0.042</td>
<td>0.013</td>
<td>Chg $D_{\text{mean}}$ per 1 unit $\text{CS}$</td>
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<tr>
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<td>0.011</td>
<td>Within-subject variance</td>
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<tr>
<td>Level 2 $U_{00}$ Intercept</td>
<td>$0.223$</td>
<td>&lt;0.001</td>
<td>0.225</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$U_{10}$ Slope</td>
<td>2.087</td>
<td>&lt;0.001</td>
<td>3.096</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$D_{\text{mean}} =$ mean diameter. SR = shear rate. VA = velocity acceleration. CS = change in circumferential stress (potential energy).
Table 3.2. *HLM estimates for effects of condition on change in diameter with shear rate.*

<table>
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<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
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</tbody>
</table>
| Intercept $\beta_{00}$ | $D_{\text{mean}}$ | 4.061 | $<0.001$ | 4.067 | $<0.001$ | *Initial $D_{\text{mean}}$*
| P1 $\beta_{10}$ | SR | 3.392$^{10^{-4}}$ | $<0.001$ | 3.192$^{10^{-4}}$ | $<0.001$ | Chg $D_{\text{mean}}$ per 1 unit SR
| P2 $\beta_{20}$ | SR*Cond | -0.372$^{10^{-4}}$ | 0.015 | -0.129$^{10^{-4}}$ | 0.564 | Condition effect
| P3 $\beta_{30}$ | VA | -0.187$^{10^{-4}}$ | $<0.056$ | 0.056 | Chg $D_{\text{mean}}$ per 1 unit VA
| P4 $\beta_{40}$ | CS | 0.029 | 0.032 | Chg $D_{\text{mean}}$ per 1 unit CS
| **Random Variance** |         |               |             |               |
| **Level 1** | $E$ | Residual | 0.013 | 0.011 | *Within-subject variance*
| **Level 2** | $U_{00}$ | Intercept | 0.223 | $<0.001$ | 0.225 | $<0.001$ | *Between-subject variance*
| | $U_{10}$ | Slope | 2.087 | $<0.001$ | 3.096 | $<0.001$ | *Between-subject variance*

Cond. = condition (0 = control, 1 = acceleration condition). $D_{\text{mean}}$ = mean diameter. SR = shear rate. VA = velocity acceleration. CS = circumferential stress (potential energy). Note: VA & CS are centered by stage for each individual.
Figure 3.1.

**Control Condition**

- Rest → 40°C Heat → 42°C Heat → Exrs 1/3sec → Exrs 2/3sec

**Acceleration Condition**

- Pre-Rest → 40mmHg Cuff → Rest → 40°C Heat → 42°C Heat → Exrs 1/3sec → Exrs 2/3sec

<table>
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<tr>
<th>Stage</th>
<th>1</th>
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</tbody>
</table>
Figure 3.2.
Figure 3.3.

A

VA = 1085 cm/s²
V_m = 7.3 cm/s
V_s = 106 cm/s
V_d = 0 cm/s

B

VA = 1230 cm/s²
V_m = 88 cm/s
V_s = 164 cm/s
V_d = 57 cm/s

C

VA = 1204 cm/s²
V_m = 7.8 cm/s
V_s = 107 cm/s
V_d = 0 cm/s

D

VA = 1609 cm/s²
V_m = 89 cm/s
V_s = 166 cm/s
V_d = 56 cm/s
Figure 3.4.

![Graph showing the relationship between Shear Rate (s⁻¹) and Velocity Accel (cm/s²) with two datasets: Control (open circles) and Acceleration (filled squares).](image-url)
Figure 3.5.
Figure 3.6.
CHAPTER IV

EVALUATION OF THE DURATION OF SHEAR STIMULUS ON

FLOW-MEDIATED DILATION$^1$

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$^1$Lee Stoner, Andrew J. Arthur and Kevin K. McCully.

To be submitted to the Journal of Applied Physiology
ABSTRACT

Flow-mediated dilation (FMD) is typically assessed by measuring the vessel diameter increase in response ischemia-induced increases in shear stress. FMD is attenuated by a number of disease states. However, FMD may also be “attenuated” if the hyperemic response is lower than expected. Peak- and time-integrated shear rates can substantially vary between-individuals. We assessed whether peak- and time integrated-shear rates independently predict FMD. We define FMD as the shear rate-diameter relationship. We hypothesized that peak shear rate does independently predict FMD. Eleven physically active, young (25±5 years) male subjects were tested. The shear rate-diameter relationship was assessed using transient and state-state increases in shear rate. For the transient condition, shear rate was increased using four durations of down-stream ischemia (2, 4, 6 & 10 minutes). For the steady state condition, forearm blood flow was manipulated using progressive local heating and handgrip exercise. Hierarchical linear modeling (HLM) was used to estimate diameter change with repeated measures of shear rate nested within each subject. Our findings indicate that peak and time-integrated shear rates both independently predict FMD. When using time-integrated shear rates as the single predictor variable, the transient condition resulted in significantly smaller diameter change for a given shear rate change (P = 0.012). However, when also specifying peak shear rate as a covariate, the difference between conditions became non-significant (P = 0.138).

INDEX WORDS: Flow mediated dilatation, flow pulsatility, arterial responsiveness, shear stress, hierarchical linear modeling.
INTRODUCTION

Flow-mediated dilation (FMD) is the auto-regulation of blood vessel size in response to flow-induced increases in shear stress. FMD is governed by the release of dilatory molecules, including nitric oxide, from the vascular endothelium (28). The vascular endothelium is an important monolayer which regulates vascular homeostasis. Endothelial dysfunction initiates atherosclerosis, the precursor to cardiovascular disease. The FMD test was developed to non-invasively assess endothelial health (11, 13). This test is accomplished by inflating a tourniquet around the forearm to a supra-systolic pressure for 4.5-5 minutes (11, 13). Release of the tourniquet results in a large transient increase in shear stress. The magnitude of FMD, expressed as the percentage increase in diameter above rest, is used to represent endothelial health. Reduced FMD is an early marker of atherosclerosis (11) and has been noted for its predictive capacity for future cardiovascular complications (40).

While FMD is certainly attenuated in a number of disease states, FMD may also be “attenuated” if the hyperemic (shear stress) response is lower than expected. Peak- and time-integrated shear rates can vary substantially between-individuals (20, 25, 42). It is therefore imperative that the magnitude of the stimulus imposed on the endothelial cells is quantified. A previous study from our laboratory suggests that the shear stimulus is best quantified by integrating the hyperemic (shear) response (42). However, other groups suggest that the peak shear response is also important in determining FMD (20, 25). There remains a need to comprehensively quantify the appropriate expression of the shear stimulus. The purpose of our study was to assess whether peak and time-integrated shear rates independently predict FMD. We define FMD as the shear rate-diameter
relationship. We hypothesized that peak shear rate does independently predict FMD in addition to time-integrated shear rate. An additional purpose was determine the likelihood of flow turbulence, and alterations to flow pulsatility, occurring during reactive hyperemia.

METHODS

Subjects and Study Design

Thirteen healthy, physically active, young male subjects were recruited. None of the subjects reported cardiovascular disease complications. The study was approved by the University of Georgia Institutional Review Board. Informed consent was obtained from the subjects after they were given a detailed description of the procedures.

The test sessions were performed between the hours of 7 am and 10 am to reduce circadian variation. 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 known vascular actions prior to testing. Subjects were excluded from the study if they were currently prescribed medications with known vascular actions. All stages of testing were performed in a climate controlled laboratory setting.

Protocol

Each subject was tested on two days (transient and steady-state conditions) separated by less than 7 days. The study outline is shown in Figure 4.1. On each day testing commenced following at least 10 minutes of quiet supine rest. Left arm brachial artery diameters and blood velocities were measured continuously during the transient condition, and during the last 30 seconds of each stage for the steady-state condition.
Flow turbulence was estimated by calculating Reynold’s number. Flow pulsatility was estimated by calculating pulsatility index.

Ischemia

A pneumatic tourniquet (Hokanson, Inc., Seattle, Washington) placed around the limb, distal to the insonated artery, was rapidly inflated (1-2 seconds) to a pressure of approximately 100 mmHg above the systolic blood pressure. Blood pressures measurements were taken on the non-imaged arm.

Indirect Local Heating

The subject’s forearm was placed into a specially constructed air-tight container. The base of the container was lined with a digital moist heating pad and an inlet was used to circulate warm air. Warming of the skin is thought to increase blood flow locally without significant systemic autonomic influence (21, 44). The forearm was heated to 40°C for 10 minutes and then to 42°C for 5 minutes. Forearm and bicep skin temperatures were monitored using fast response temperature probes (TSD202B, Biopac Systems, Inc., Goleta, California).

Forearm Exercise

Two stages of isometric rhythmic handgrip exercise were performed using a handgrip ergometer. For each stage the subject was asked to squeeze to 10% of their maximal voluntary contraction (MVC). Each stage lasted 4 minutes. For stage one the subject contracted once every 3 seconds. For stage two the subject contracted twice every 3 seconds. Low intensity handgrip exercise of a small muscle group was chosen to minimize systemic autonomic responses. Subjects were encouraged to perform the exercise with minimal bicep activity. Bicep electrical activity was monitored using electromyography (EMG 100C, Biopac Systems, Inc., Goleta, California). Force output
was captured continuously (MP100, Biopac Systems, Inc., Goleta, California) and visual feedback was provided to the subject. Velcro straps were used to fasten the forearm.

**Ultrasound Diameter Measurements**

High-resolution B-mode ultrasound measurements were made using a GE 400CL duplex color Doppler unit (GE Medical, Milwaukee, Wisconsin) equipped with a 7-13 MHz linear array transducer (LA39). Ultrasound global (acoustic output, gain, dynamic range, gamma, and rejection) and probe-depandant (zoom factor, edge enhancement, frame averaging, and target frame rate) settings were standardized. Care was taken to ensure that the vessel clearly extended across the entire [un-zoomed] imaging plane to minimize the likelihood of skewing the vessel walls. The image was adjusted until the vessel walls appeared thickest. The image was then zoomed to focus on the center of the vessel. The image was comprised of 400*400 pixels, an area of 16*16 mm with a pixel resolution of 0.04*0.04 mm. Image focus was maintained using a specialized probe holding device. The probe holding device allowed fine adjustments to probe positioning and was used to elevate the probe from the skin to avoid pressure being applied to the artery.

**Diameter analysis**

Moving Picture Experts Group-2 (MPEG-2) recordings were captured using a Dell Laptop PC equipped with a with video capture device (ADS technologies, Cerritos, California). Video files collected at 30 frames/second were converted into Joint Photographic Experts Group (JEPG) images and subsequently used to make 30 diameter measurements/second. JPEG images provide comparable accuracy for ultrasound image measurements compared to the Digital Image and Communications in Medicine
Two 10 second movies were taken for each stage of testing. Subjects were asked to hold their breath during movie capture. Images were measured offline using semi-automated edge-detection software custom written to interface with the LabVIEW data acquisition platform (version 8.1, National Instruments, Austin, Texas) (37, 43). Custom written Excel Visual Basic code was used to fit peaks and troughs to diameter waveforms in order to calculate diastolic, systolic, and mean diameters. Mean diameters were used for analysis. The within-session SEM for the described set-up is 0.046 mm. Between-day coefficients of variation are 2.4-2.7% (42).

**Blood Velocities**

Sonication angle was kept constant between 45-65° and the sample volume included most of the vessel. Blood velocities were calculated using the advanced vascular package supplied with the GE 400CL ultrasound machine. A custom written Optical Character Reading (OCR) package written in LabVIEW was used to capture blood velocities for each cardiac cycle. When more than two values were recorded for a given second, the average was computed. Obvious outliers were removed and missing values were replaced using linear interpolation. In our laboratory, between-day coefficients of variation for peak velocity measurements are 14.6% (42).

**Shear Rate**

The 30 diameters measurements/second were aggregated to 1/second and synchronized with blood velocities. Shear rates were calculated as the product of:

\[
\text{Shear Rate} \ (s^{-1}) = (8 \times \text{mean blood velocity}) \ / \ \text{diameter}
\]
Time averaged maximum blood velocities were used to calculate shear rate. Time averaged maximum velocity is the average of the highest velocities throughout the cardiac cycle. Our ultrasound machine more reliably calculates these velocities when compared to time averaged mean blood velocities (32).

Seven shear rate expressions were calculated: peak, change (peak-cuff), integrated over 10, 20, 30, and 40 seconds, and integrated to peak diameter time. Hyperemic blood velocity acceleration was also estimated:

\[
\text{Hyperemic Acceleration (cm/s}^2\) = \frac{\text{peak mean velocity} - \text{cuff mean velocity}}{\text{time to peak}}
\]

Peak mean velocity is the peak mean velocity during reactive hyperemia. Cuff mean velocity is the average mean velocity during the last 30 seconds of ischemia. Time averaged maximum blood velocities were used to represent mean velocities.

*Pulsatility Index*

Pulsatility index was calculated second-by-second:

\[
\text{PI} = \frac{\text{peak systolic velocity} - \text{minimum diastolic velocity}}{\text{mean velocity}}
\]

Time averaged maximum blood velocities were used to represent mean velocities.
Reynold’s Number

Reynold’s number was calculated second-by-second:

\[ \text{Re} = \frac{vd}{\rho / \eta} \]

where \( v \) = peak systolic velocity, \( d \) = diameter, \( \rho \) = density, and \( \eta \) = viscosity.

Density was assumed to be 1060 kg/m\(^3\), and viscosity 0.0035 Pa \( \cdot \) s.

Blood Pressures

Blood pressures were continuously measured on the right (non-imaged) arm using a finger photo-plethysmographic device (Finapress 2300, Ohmeda, Englewood, Colorado) interfaced to a Biopac data acquisition system (MP100, Biopac Systems, Inc., Goleta, California). To ensure the Finapress was correctly calibrated, the systolic and diastolic blood pressure values were checked against recordings from a semi-automated blood pressure device (Datascope Accutor 3, Montvale, New Jersey). If systolic and diastolic measurements did not agree within 5 mmHg, the finger cuff was adjusted until the two devices agreed (41).

Statistics

Descriptive data for each stage of testing are expressed as means (±SD). Between-condition resting measurements were compared using 2-tailed dependant student’s \( t \) tests. The primary study outcomes were analyzed using hierarchical linear modeling (HLM) with the HLM6 (Scientific Software International, Inc., Lincolnwood, Illinois) statistical package. A key application of HLM relates to the capacity to account for correlated measures by recognizing the nested data structure (36), i.e., repeated measures nested
within each subject. This approach models different patterns of growth trajectories by allowing for the intercepts (initial diameter) and slopes (shear rate-diameter) to randomly vary (36). Briefly, HLM was used to compare the shear rate - diameter relationship for steady state vs. transient shear rate conditions. Repeated measures (level-1) were nested within each subject (level-2). The final within-subject (level-1) and between-subject models were specified:

**Level-1 Model**

\[
Y_{si} = \pi_{0i} + \pi_{1i}(SR_{40_{si}}) + \pi_{2i}(SR_{40_{si}} \times Cond_{si}) + \pi_{3i}(SR_{peak_{si}}) + e_{si}
\]

where \(\pi_{0i}\) (intercept) represents diameter for person \(i\) when \(SR_{40_{si}} = 0\), where \(SR_{40}\) = shear rate, \(\pi_{1i}\) (slope) represents change in diameter per 1 unit shear rate, i.e., \(FMD_{i}\), \(Cond\) represents a dummy coded variable to identify steady-state (0) and transient (1) shear conditions, and \(SR_{peak}\) represents grand-mean centered peak shear rate. The intercept and slopes specified at level 1 become outcomes at level 2.

**Level-2 Model**

\[
\begin{align*}
\pi_{0i} &= \beta_{00} + r_{0i} \\
\pi_{1i} &= \beta_{10} + r_{1i} \\
\pi_{2i} &= \beta_{20} \\
\pi_{3i} &= \beta_{30}
\end{align*}
\]
where \( r_{0i} \) and \( r_{1i} \) are the unique increments associated with individual \( i \), indicating that the individual intercepts and slopes were allowed to randomly vary.

Level 2 outcomes were used for hypothesis testing. Two HLM models were estimated to test the hypothesis. Model one used only the transient data set. Model two used the combined (transient + steady-state) data set. Each of these models were initially specified as unconditional, i.e., no predictor variables. Additional predictor variables were subsequently specified within sub-models. Statistical significance was defined as \( p < 0.05 \).

**Model 1: Peak vs. integrated shear rates.** Initially, a fully unconditional model (no predictors) was specified. Each shear rate expression was then independently regressed against peak diameters. The percentage within-subject (residual) variance explained by each explanatory variable was calculated. The shear rate expression which explained most within-subject variance was used for subsequent analysis. The study hypothesis was accepted if peak shear rate significantly predicted change in diameter when specified in addition to the best-fit integrated shear rate expression.

**Model 2: Transient vs. steady-state shear rates.** The interaction term SR*Condition was specified at level-1 to determine whether FMD (i.e., shear rate-diameter relationship) significantly differed for transients vs. steady-state conditions. Peak shear rates were then grand-mean centered and specified as a covariate to determine whether this variables explain differences in FMD between conditions. The study hypothesis was accepted if peak shear rate explained the difference in FMD between conditions. Note: the peak shear rates are equal to integrated shear rates for the steady state conditions.
Estimated reliability. The estimated reliability is defined as the ratio between the level-2 variance component and the sum of the level-2 and level-1 components, with the latter divided by the number of observations (36). Poor reliability (below 0.10) would render the data incapable of identifying relationships between variables for the given population sample size (36). The relationship between the slope (FMD) and the intercept was calculated as the intra-class correlation coefficient between intercept and slope random variance components.

RESULTS

Analysis was conducted on eleven of the thirteen subjects. One subject was omitted out due to poor B-mode Ultrasound image quality, another subject due to technical difficulties. For the remaining eleven subjects the average age was 25±5 years, height 176±6 cm, and weight 79±11 kg. Subjects exercised an average of 5±4 times/week for 59±26 minutes/session. Average systolic, diastolic and mean blood pressures were 120±10 mmHg, 70±7 mmHg, and 89±8 mmHg, respectively. Blood pressures did not significantly differ between conditions.

Model 1: Peak vs. Time-Integrated Shear Rates.

Figure 4.3 shows individual and average shear rate and diameter responses to the different durations of ischemia. Increasing the cuff duration resulted in a progressive increase in both the peak and the duration of the shear rate and diameter responses. Figure 4.4 shows peak diameters plotted against shear rate integrated over 40 seconds. Vessel diameters in particular demonstrate a large amount of variation. The majority of this variation can be explained by two factors. Firstly, resting diameters ranged from 3.28 to 4.78 mm. Individuals with larger initial diameters would be expected to have greater
absolute FMD responses. Secondly, the standard deviations are misleading. The calculation of standard deviations forces the continuous data to become categorized according to stage. The shear rate responses varied notably between subjects, this would influence the magnitude of FMD. This explains why, when specifying shear rate integrated over 40 seconds, that despite the variation the reliabilities for the intercept and slope were 0.99 and 0.76, respectively. These results signify that despite differences in initial diameter, change in diameter can be adequately predicted by shear rate. These results also indicate that there was adequate signal in the data to specify additional predictor variables and detect the resultant effect.

Table 4.1 shows the final two sub-models run to test the study hypothesis. Each shear rate expression was independently regressed against peak diameters using HLM analysis. Figure 4.5 shows the within-subject (residual) variance explained for each model. Variation explained improved with increasing duration of shear rate integration, leveling off at approximately 30 seconds. However, shear rates integrated to peak diameter time did not explain as much variation as shear rates integrated over 40 seconds. The addition of peak shear rate to 40 seconds integrated shear rate resulted in a small but significant ($P = 0.005$) improvement in variance explained. The addition of velocity acceleration and circumferential stress predictors improved the reliability of the slope (FMD) to 0.81. The purpose of this study was to explain within-subject (level-1) variance, i.e., predict FMD for each subject, not to explain differences between-subjects (level-2). We did not expect to decrease between-subject variance. However, it is worth noting that the between-subject random variance for intercept and slope parameters
remained significant. The addition of between-subject subject predictors, e.g., resting diameter, age, weight etc., may have improved the capacity to predict FMD.

**Model 2: Transient vs. Steady-State Shear Rates.**

Three HLM sub-models were specified. Table 4.2 shows sub-models two and three. Figure 4.6 shows the estimated HLM model fit with and without peak shear specified as a covariate. The initial sub-model specified integrated shear rate (40 seconds) as the only predictor variable. The subsequent sub-model specified SR*Condition to assess whether the shear rate-diameter relationship was different for transient vs. steady-state shear rates. FMD was 39% (P = <0.001) slower for the transient (β = 4.25\texttimes10^{-4}; 95%CI: 3.35\texttimes10^{-4}, 5.15\texttimes10^{-4}) vs. steady-state (β = 2.59\texttimes10^{-4}; 95%CI: 3.96\texttimes10^{-4}, 6.88\texttimes10^{-4}) condition. The third sub-model specified peak shear rate as a grand-mean centered covariate. The difference between steady-state (β = 5.42\texttimes10^{-4}; 95%CI: 3.96\texttimes10^{-4}, 6.88\texttimes10^{-4}) and transient (β = 4.54\texttimes10^{-4}; 95%CI: 3.61\texttimes10^{-4}, 5.47\texttimes10^{-4}) FMD slopes became non-significant (P = 0.138).

**Secondary Flow Characteristics**

Table 4.3 characterizes the secondary flow characteristics measured. Figure 4.2 shows representative velocity waveforms taken from one subject at rest, immediately post 10 minutes ischemia, and 10 seconds post 10 minutes ischemia. At rest, the spectral waveform is characterized by a narrow range of frequencies, with the energy concentrated on the highest frequencies. Immediately post-ischemia, a wide range of frequencies is seen, with energy spread more evenly across the range of frequencies. The *spectral broadening* may indicate a more parabolic flow profile, or the presence of turbulent flow. Figure 4.7a shows the potential for turbulence during reactive hyperemia.
Post 4 minutes ischemia, the Reynold’s number is above the critical threshold for turbulence for the first 12 seconds post-ischemia. Figure 4.7b shows that flow pulsatility decreases following ischemia.

**DISCUSSION**

We found that peak and time integrated-shear rates both independently predict FMD. Two lines of evidence support this statement: 1) The shear rate-diameter relationship was assessed using ischemia as the sole modality to increase shear rate. Shear rates integrated over 40 seconds explained the most variation for diameter change, i.e. FMD. However, peak shear rate explained an additional small, albeit significant, portion of variation for diameter change. And, 2) The shear rate-diameter relationship was assessed using transient (ischemia) and steady-state (forearm heating & handgrip exercise) modalities to increase shear rate. When using time-integrated shear rates as the only predictor variable, the transient condition resulted in significantly smaller diameter change for a given shear rate change. However, when also specifying peak shear rate as a covariate, the difference between conditions became non-significant.

*Peak vs. Time-Integrated Shear Rates*

Shear rates integrated for 40 seconds post-ischemia explained a greater portion of variation for change in diameter compared to peak shear rates. This findings may be explained by: 1) The use of a single peak measurement is more prone to the influence of physiological variance (e.g., respiratory cycle) and measurement error. Or., 2) Peak reactive hyperemic responses do not as adequately define the stimulus for vasodilatation (25). However, the addition of peak shear rate to the time-integrated shear rate was significant. This suggests that peak shear rate may be an additional important
independent predictor of FMD. It is worth noting that while peak shear positively predicted peak diameter when regressed independently, the addition of 40 seconds integrated shear rate led to a negative relationship between peak shear rate and peak diameter. A greater peak shear rate for a given integrated shear rate is indicative of a more transitory hyperemic response. A less sustained increase in shear rate would result in a lower stimulus mechanotransduced to the endothelial cells.

Shear rates integrated to the time of peak diameter did not explain as much variation as shear rates integrated over 40 seconds. Our data indicate that the bulk of the hyperemic response occurs during the initial 30-40 seconds following ischemia. The peak diameters response occur at approximately 35-55 seconds. Integrating shear rate to peak diameter time may not have explained as much variation as shear rate integrated to 40 seconds due to: 1) The bulk of the shear stimulus may already have passed. Or, 2) measurement error may be introduced when manually identifying the time of peak diameter.

*Transient vs. Steady-State Shear Rates*

When regressing time-integrated (40 seconds) shear rates against peak diameters, FMD was 39% slower for transient vs. steady-state increases in shear rate. However, when co-varying for peak shear rates, the difference in FMD slopes between conditions became non-significant. These findings may be explained by: 1) The different dilatory mechanisms recruited for transient vs. steady-state increases in shear rate. Or, 2) The shear stimulus is underestimated for transient increases in shear rate.

The mechanisms regulating vascular tone may be dependant on the duration of the shear stimulus. Nonetheless, the endothelium is still thought to primarily govern
vasodilation under steady-state conditions. Studies have shown that hand warming has no effect on brachial artery diameter when flow is not allowed to rise (20, 29, 35). Furthermore, pharmacological blockade of the autonomic nervous system has no effect on radial artery FMD in response to hand warming (29), consistent with animal studies showing that FMD is preserved after surgical or pharmacological denervation (18, 26).

Endothelial cells regulate vascular tone through the release of several vasodilators, including nitric oxide (22), prostaglandins (prostacyclin) (23), and a putative endothelium-derived hyper-polarization factor (9). The relative importance of these vasodilators appears to be dependent on the duration of the shear stimulus. Both nitric oxide and prostacyclin demonstrate biphasic responses to the onset of flow, with an initial rapid release followed by more steady production (15, 16, 24, 27, 29). The rapid initial production of nitric oxide is thought to be calcium/calmodulin-dependent in contrast to the sustained phase, which appears to be independent of these messengers (27, 29). Further study is needed in order to determine whether these potential mechanistic differences can explain the greater FMD with saw with steady-state vs. transient flow conditions.

We estimated shear rate based on Poiseuille’s law \( \left( \frac{8v}{d} \right) \), which assumes that: 1) blood is a Newtonian fluid, 2) blood flows through a rigid tube, and 3) the velocity profile is parabolic. First, blood is non-Newtonian, though the effect of the non-Newtonian behavior does not appear to be pronounced in large arteries (12). Second, blood vessels are distensible, increases in the arterial cross-sectional during the cardiac cycle can reduce wall shear rate approximately 30% as compared with rigid tubes (14). Third, in order for the profile to be parabolic, blood flow needs to be laminar. Beyond the
critical Reynold’s number of 2,000 flow becomes turbulent, leading to a blunt flow profile. One result is that Poiseuille’s law is no longer valid. We found that even 2 minutes ischemia resulted in approximately 2 seconds of potentially turbulent flow. This finding was supported by the increases in spectral broadening and the decreases flow pulsatility that we saw following ischemia. Collectively, these observations suggest that the assumptions for calculating shear rate based on Poiseuille’s law are violated during reactive hyperemia.

Conclusions

We demonstrated that time-integrated and peak shear rates independently predict FMD. This finding was supported by the similar magnitudes of FMD we saw in response to transient (ischemia) and steady-state (heating & exercise) increases in shear rate when accounting for both the integrated and peak shear stimulus. We also found that assumptions for calculating shear rate based on Poiseuille’s law may be violated during reactive hyperemia. Further research is warranted to determine whether steady-state increases in shear rate can be used to predict cardiovascular events.
REFERENCES


FIGURES

Figure 4.1. *Protocol for steady-state and transient conditions.* Steady state: For the heating conditions only the forearm was heated. For the exercise conditions the forearm remained heated to 42°C. Exercise was performed at 10% MVC. Total testing time was 24 minutes. Transient: 5min recovery was allowed between each condition. Total testing time was 43 minutes.

Figure 4.2. *Representative velocity waveforms taken from one subject at rest and 10 seconds post 10 minutes ischemia.* The resting waveform is characterized by a narrow range of frequencies, with the energy concentrated on the highest frequencies. Post-ischemia a wide range of frequencies is seen, with energy spread more evenly across the range of frequencies. The *spectral broadening* may indicate a more parabolic flow profile, or the presence of turbulent flow.

Figure 4.3. *Mean diameters and shear rates (A + B).* Diameters and shear rates post ischemia for 1 subject (C + D). Horizontal lines represent resting values.

Figure 4.4. *Mean shear rates plotted against peak diameters for transient and steady-state conditions.* Shear rate for the transient condition are integrated over 40s post-ischemia. Error bars show representative standard deviations.
Figure 4.5. *Within-subject variance explained by shear rate expression.* Each shear rate expression was independently regressed against peak diameters using HLM. Velocity acceleration was co-regressed with peak shear rates.

Figure 4.6. *HLM model estimates for shear rates regressed against peak diameters.* HLM models *without* (A) and *with* (B) peak shear being co-varied are shown.

Fig 4.7. *Change in Reynold’s number (B) and pulsatility index (B) during reactive hyperemia.* Figure 7A shows the potential for turbulent flow occurring during reactive hyperemia. Figure 7B shows that flow pulsatility decreases following ischemia.
Table 4.1. *HLM estimates for change in diameter with shear rate.*

<table>
<thead>
<tr>
<th></th>
<th>SR(_{40})</th>
<th></th>
<th>SR(<em>{40}) + SR(</em>{\text{peak}})</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est.</td>
<td>p</td>
<td>Est.</td>
<td>p</td>
</tr>
<tr>
<td><strong>Fixed Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (\beta_{00}) D(_{\text{mean}})</td>
<td>4.103</td>
<td>&lt;0.001</td>
<td>4.112</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P1 (\beta_{10}) SR(_{40})</td>
<td>2.532(10^{-4})</td>
<td>&lt;0.001</td>
<td>2.532(10^{-4})</td>
<td>&lt;0.001  Chg D(<em>{\text{mean}}) per 1 unit SR(</em>{40})</td>
</tr>
<tr>
<td>P2 (\beta_{20}) SR(_{\text{peak}})</td>
<td>-0.762(10^{-4})</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Random Variance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1 (E) Residual</td>
<td>0.003</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 (U_{00}) Intercept</td>
<td>0.186</td>
<td>&lt;0.001</td>
<td>0.188</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U_{10}) Slope</td>
<td>0.606</td>
<td>&lt;0.001</td>
<td>0.674</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(D_{\text{peak}}\) = peak diameter. \(SR_{40}\) = shear rate integrated over 40 seconds post-ischemia.

\(SR_{\text{peak}}\) = peak shear rate post-ischemia.
Table 4.2. *HLM estimates for change in diameter with steady-state vs. transient shear rate.*

<table>
<thead>
<tr>
<th></th>
<th>SR(_{40})</th>
<th>SR(<em>{40}) + SR(</em>{\text{peak}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est.</td>
<td>p</td>
</tr>
<tr>
<td>Fixed Effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercep</td>
<td>(\beta_{00})  D(_{\text{mean}}) 4.089  0.001</td>
<td>3.941  0.001</td>
</tr>
<tr>
<td>P1</td>
<td>(\beta_{10})  SR(_{40})  4.247(10^{-4})  0.001</td>
<td>5.416(10^{-4})  0.015</td>
</tr>
<tr>
<td>P2</td>
<td>(\beta_{20})  SR*Cond -1.655(10^{-4})  0.012</td>
<td>-0.880(10^{-4})  0.138</td>
</tr>
<tr>
<td>P3</td>
<td>(\beta_{30})  SR(_{\text{peak}})  -1.240(10^{-4})  0.037</td>
<td></td>
</tr>
<tr>
<td>Random Variance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>E</td>
<td>Residual 0.016  0.015</td>
</tr>
<tr>
<td>Level 2</td>
<td>(U_{00})  Intercept 0.233  0.001</td>
<td>0.232  0.001</td>
</tr>
<tr>
<td></td>
<td>(U_{10})  Slope 0.725  0.024</td>
<td>0.784  0.016</td>
</tr>
</tbody>
</table>

\(\text{Cond.} =\) condition (0 = stead-state, 1 = transient). \(D_{\text{peak}}\) = peak diameter. \(SR_{40}\) = shear rate integrated over 40 seconds post-ischemia. \(SR_{\text{peak}}\) = peak shear rate post-ischemia. Note: \(SR_{\text{peak}}\) is grand-mean centered.
Table 4.3. *Flow characteristics post-ischemia.*

<table>
<thead>
<tr>
<th>Isch. min</th>
<th>D_{TTP} s</th>
<th>SR_{TTP} s</th>
<th>SR_{chg} s</th>
<th>Accel s^{-1}</th>
<th>SR_{40} s^{-1}</th>
<th>PI_{40} s</th>
<th>Re_{40} s</th>
<th>Re_{time} s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>231 (98)</td>
<td>8 (4)</td>
<td>884 (388)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>52 (26)</td>
<td>5 (3)</td>
<td>1545 (253)</td>
<td>469 (551)</td>
<td>837 (227)</td>
<td>3 (1)</td>
<td>1501 (175)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>4</td>
<td>35 (9)</td>
<td>8 (3)</td>
<td>2050 (242)</td>
<td>315 (160)</td>
<td>1154 (177)</td>
<td>2 (1)</td>
<td>1784 (188)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>6</td>
<td>42 (11)</td>
<td>9 (2)</td>
<td>2252 (400)</td>
<td>272 (89)</td>
<td>1373 (265)</td>
<td>2 (0)</td>
<td>1914 (225)</td>
<td>18 (13)</td>
</tr>
<tr>
<td>10</td>
<td>55 (19)</td>
<td>11 (2)</td>
<td>2537 (375)</td>
<td>247 (71)</td>
<td>1781 (310)</td>
<td>1 (0)</td>
<td>2214 (236)</td>
<td>36 (16)</td>
</tr>
</tbody>
</table>

D_{TTP} = time to peak diameter. SR_{TTP} = time to peak shear rate. SR_{chg} = change in shear rate from pre- to post-ischemia. Accel = hyperemic acceleration (SR_{chg} / SR_{TTP}). SR_{40} = shear rates integrated over 40s post-ischemia. PI_{40} = pulsatility integrated over 40 seconds post-ischemia. Re_{40} = Reynold’s number integrated over 40 seconds post-ischemia. Re_{time} = time flow turbulent spent turbulent (Re > 2000) post-ischemia.
Figure 4.1.

**Transient (ischemia) Shear Rate Condition**

Rest → 2min → 4min → 6min → 10min

**Steady-State Shear Rate Condition**

Rest → 40°C Heat → 42°C Heat → Exrs 1/3sec → Exrs 2/3sec
Figure 4.2.
Figure 4.3.

A. Individual Example

B. Individual Example

C. Mean Data

D. Mean Data
Figure 4.4.
Figure 4.5.
Figure 4.6.

**Without SRpeak**

- Diameter (mm)
- Shear Rate (s⁻¹)
- P = 0.012

**With SRpeak**

- Diameter (mm)
- Shear Rate (s⁻¹)
- P = 0.138
Figure 4.7.

A

Critical threshold for flow turbulence

B

Rest
2 min
4 min
6 min
10 min

Rest
2 min
4 min
6 min
10 min

Rest
2 min
4 min
6 min
10 min

Rest
2 min
4 min
6 min
10 min
CHAPTER V

SUMMARY AND CONCLUSIONS

Cardiovascular disease remains the leading cause of morbidity and mortality in western nations. Endothelial dysfunction precedes atherosclerosis and subsequent cardiovascular disease. The flow-mediated dilation (FMD) test is a non-invasive tool used to assess endothelial health. However, the usefulness of this tool is limited if FMD is not normalized to its stimulus. Shear stress is the established stimulus for FMD. Shear stress can be calculated as the product of shear rate and blood viscosity. Doppler ultrasound can be used to estimate shear rate. Two potential limitations are inherent to estimates of shear rate: 1) The assumption that mean blood velocity adequately explains the shear stimulus. Pulsatile blood flow results in endothelial being exposed to two distinct shear stimuli during the cardiac cycle: a large rate of change (velocity acceleration) in shear at the onset of flow, followed by steady shear. Velocity acceleration may be an important independent stimulus for FMD. And, 2) FMD is typically tested using 5 minutes of ischemia. Ischemia elicits large but very transient increases in shear stress. There remains a need to identify whether peak- and time-integrated shear rates independently predict FMD.

The purpose of study one was to determine the importance of velocity acceleration to the FMD phenomena. FMD was measured prior to and following induced increases in velocity acceleration. Mean blood velocity was kept constant between conditions. The purpose of study two was to assess whether peak- and time integrated-shear rates independently predict FMD. Findings from the first study indicate that that induced increases in velocity acceleration attenuate FMD in physically active, healthy
young males. FMD was attenuated despite no alteration in endothelial function. This finding suggests that the shear stimuli may not be appropriately expressed as a linear function of mean blood velocity.

The second study demonstrated that peak- and time integrated-shear rates both independently predict FMD. Two lines of evidence support this statement. Firstly, the shear rate-diameter relationship was assessed using ischemia as the sole modality to increase shear rate. Shear rates integrated over 40 seconds explained the most variation for diameter change, i.e. FMD. However, peak shear rate explained an additional small, albeit significant, portion of variation for diameter change. Secondly, the shear rate-diameter relationship was assessed using transient (ischemia) and steady-state (forearm heating & handgrip exercise) modalities to increase shear rate. When using solely time-integrated shear rates as the only predictor variable, the transient condition resulted in significantly smaller diameter change for a given shear rate change. However, when also specifying peak shear rate as a covariate, the difference between conditions became non-significant.

**Recommendations**

1. **Normalize FMD to the shear stimulus.** FMD is dependent not only on endothelial function, but also the magnitude of the shear stimulus. Especially in response to ischemia, the stimulus can vary quite substantially between individuals, or within individuals undergoing therapy.

2. **Pay attention to velocity acceleration.** The current findings support in vitro studies demonstrating velocity acceleration to moderate endothelial function. Mean blood velocity may not adequately define the shear stimulus. This recommendation may be
particularly important to patient groups with known alterations in the shape of their velocity profile

3. **Pay attention to the peak shear response.** Ischemia results in a sharp peak shear response. Peak shear may be an important component of the shear stimulus in addition to time-integrated shear.

4. **Pay attention to the duration of the shear stimulus.** Steady-state shear was found to result in greater diameter change responsiveness than transient shear. This suggests that the duration of the shear stimulus may be important to the subsequent FMD. With ischemia the duration of hyperemia may vary substantially between individuals. For example, with spinal cord injury the duration of reactive hyperemia is increased. Different vascular beds also exhibit different duration of reactive hyperemia.

**Future Direction**

A number of important questions need to be determined:

1. Does FMD in response to steady-state shear stress predict CVD as well as in response to transient increases in shear?

2. Can shear stress adjustments to the blood velocity profile be used to improve the reliability of FMD measurements?

3. What is the most important: the particular vasodilators released in response to shear stress? Or, the magnitude of the FMD response?
REFERENCES


81. Gnasso A, Carallo C, Irace C, Spagnuolo V, De Novara G, Mattioli PL, and Pujia A. Association between intima-media thickness and wall shear stress in


112. **Johnson J, M., O'Leary DS, Taylor WF, and Kosiba W.**

113. **Katz SD, Krum H, Khan T, and Knecht M.**

114. **Kellogg DL, Jr., Crandall CG, Liu Y, Charkoudian N, and Johnson JM.**

115. **Kellogg DL, Jr., Liu Y, Kosiba IF, and O'Donnell D.**


117. **Klymkowsky MW, and Parr B.**


126. **Lee BK, Kwon HM, Hong BK, Park BE, Suh SH, Cho MT, Lee CS, Kim MC, Kim CJ, Yoo SS, and Kim HS.** Hemodynamic effects on atherosclerosis-


