2001

The time-course of vascular adaptations following localized short term exercise training

Jason David Allen

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THE TIME-COURSE OF VASCULAR ADAPTATIONS FOLLOWING LOCALIZED
SHORT-TERM EXERCISE TRAINING

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Kinesiology

By

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December 2001

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Michael Welsch for his friendship, guidance, and dedication to my development, throughout my program of study. He has served as my major professor and chair of my dissertation committee. I feel extremely fortunate to be counted as one of his students. I would also like to thank Drs. Steven Kamerling, Arnold Nelson, Changaram Venugopal, Grover Waldrop, and Robert Wood for serving on my committees, and sharing their insight, knowledge and time. Throughout, your help has always been constructive, beneficial, and appreciated.

Thank you to my colleagues who have helped me transport subjects, collect, analyze, and interpret data and write papers and presentations. Particular thanks go to Jonathan Hoy and Micah Cating for bailing me out at crunch times on many occasions and to Tracie Parish and Lisa Johnson for being my friends and making work much more fun and to my friends outside school who make having fun much more fun.

Finally, my parents David and Ruth who were always there to offer me support both emotionally, but more often than not financially.

PREFACE

This dissertation documents a literature review and 3 experiments conducted in the exercise physiology discipline of the Department of Kinesiology at Louisiana State University and Pennington Biomedical Research Center, Baton Rouge, Louisiana.

Chapter 1 outlines the issues under investigation and a brief rationale for the experiments presented in subsequent chapters. Chapter 2 outlines an experiment into the stability and reproducibility of a technique developed to assess brachial artery flow-mediated dilation and was conducted over the course of 1997 and 1998. This paper is under review for publication by the journal *Medicine and Science in Sports and Exercise*. Chapter 3 outlines a cross-sectional study conducted in 1999, entitled “Influence of age and normal plasma fibrinogen levels on flow mediated dilation in healthy adults”. This was published in the *American Journal of Cardiology* in 2000. Chapter 4 outlines a prospective interventional study conducted in 2001 to investigate the time course of vascular adaptations following localized short-term exercise training. Chapter 5 is a summary of the work in the preceding 3 chapters and an attempt to give a contextual meaning to the studies results and conclusions.

Appendix A is an extensive review of literature, completed over the course of 2000, and entitled “Human circulation: The peripheral vascular system and its regulation and adaptation to physical stress in health and disease”. A section from this paper is being adapted as a review paper for the *Journal Sports Medicine*.

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ABSTRACT

This is a series of 3 experiments in the area of vasoreactivity. The first study investigated the stability and reproducibility of brachial artery flow-mediated dilation (BAFMD). Twenty-six healthy volunteers underwent 3 scans on 2 days, performed by 2 ultrasonographers, and analyzed by 2 readers. All subjects were tested between 7 and 11 am after refraining from food and exercise. Average baseline diameter was 3.48 ± 0.53 mm, which increased to 3.71 ± 0.57 mm ($6.58 \pm 4.15\%$) at peak dilation. ICC's for days, testers and readers were 0.9188, 0.9394, and 0.8982 respectively. To detect a difference in vasoreactivity of 60% (two-tailed), e.g. 5% vasodilation versus 8% vasodilation, at 90% power, 23 and 10 subjects would be required for cross-sectional and pre-post designs, respectively. These data indicate adequate stability and reproducibility of the BAFMD technique under controlled conditions.

The second study investigated the relation between BAFMD and normal plasma fibrinogen (PF) in 30 non-smoking, healthy, volunteers (41 ± 12 yr.; range: 22-57). Results indicated a 6.08% increase (range: -3.58 to 17.48%) in BAFMD. Correlation analysis indicated significant inverse relationships for BAFMD and age $r = -0.417$ ($p = 0.02$), and for BAFMD and PF $r = -0.56$ ($p = 0.001$). Stepwise multiple regression including PF, age, total cholesterol and blood pressure revealed PF as the predominant predictor for BAFMD with 31% of the variance accounted for by $\text{BAFMD} = 22.61 - (0.05836 * \text{PF})$, with no other variables entered.

The final study examined the time-course of changes in BAFMD in response to a localized exercise training protocol. Fourteen healthy males (age: 26 ± 5.7) underwent BAFMD assessment prior to, during and following 4 weeks (5 days per week) of 60% maximal voluntary contraction handgrip training of the non-dominant arm.

Following training, paired t-tests revealed a 62% increase in BAFMD for the trained arm ($6.2 \pm 3.0\%$ to $10 \pm 4.1\%$), compared to baseline ($p=0.0004$), and a 100% difference ($10 \pm 4.1\%$ vs. $4.6 \pm 4.4\%$) between arms ($p=0.0001$). An increase in BAFMD ($6.2 \pm 3.0\%$ to $8.25 \pm 2.4\%$), was evident following 4 days of training ($p=0.015$), although individual participant variability reduced the statistical power of these findings. There were no changes in hemodynamic, autonomic and hematological variables, suggesting the improvement was a locally mediated process.

CHAPTER 1. INTRODUCTION

The purpose of this introductory chapter is to provide a brief insight into the underpinning theme of the doctoral work herein, and to provide structure to the subsequent document chapters. Chapters 2 through 4 are articles for publication and therefore individually contain brief reviews of the literature to place them in context. Additionally, a substantial review of the pertinent literature is provided in appendix A.

The circulatory system is designed to cope with a variety of situations and adapts in numerous ways to ensure optimal perfusion of the working tissues. The incredibly complex interplay of cardiovascular control mechanisms such as central, local, and reflex interactions render it impossible to examine this system in its entirety. It is therefore prudent to focus on a specific area within the whole circulatory system and examine direct influences upon it as of primary concern. Consequently, the focus of this work is the peripheral vascular system and its regulation and adaptation to physical stress in health and disease states. More specifically, the vascular responses of the brachial artery to a hyperemic blood flow stimulus.

The initial step into the investigative process was the development of an easily administered technique to provide a possible insight into the health of the cardiovascular system. Noninvasive evaluation of flow-mediated dilation of the brachial artery (BAFMD) appeared to be an good model to initially develop because it has been suggested as a potential means of identifying patients with early atherosclerosis[1, 2]. The theoretical basis for the study of flow-mediated dilation is the landmark observation by Furchott and Zawadzki [3] demonstrating the obligatory role of the endothelium and endothelium-derived relaxing factors in modulating vascular tone. Additionally, Anderson et al., and Takase et al., demonstrated a close relationship between the vasoactivity of the coronary and brachial arteries [4, 5]. Consequently, the evaluation of BAFMD

has been utilized as a non-invasive measure of vasoreactivity in many studies. The general consensus of these studies indicates reduced BAFMD in the presence of independent cardiovascular risk factors. For example, impaired BAFMD has been linked with cigarette smoking [6], hypertension [7, 8], hypercholesterolemia [9-11], hyperhomocystinemia [12], diabetes mellitus [13-16], and aging [8, 17].

Many of these studies utilize external ultrasound imaging devices with high-frequency linear array transducers to obtain an image of the brachial artery at rest and following a period of forearm occlusion, with the percent change in vessel diameter used as a marker of vascular function. Clearly, the appeal for using such a non-invasive, relatively painless, technique to possibly identify early/pre-clinical manifestations of vascular abnormalities is enormous.

Despite this the stability and reproducibility of this technique are not yet clear. Therefore the goal of the initial dissertation study was to establish the stability and reproducibility of BAFMD following 5 minutes of forearm occlusion and produce power calculations to aid in clinical trial design.

A second step in the research process was to investigate if the BAFMD technique would be able to discriminate between groups with different cardiovascular risk in a cross-sectional study design. Plasma fibrinogen was chosen as a variable to investigate for two reasons. Primarily, elevated plasma fibrinogen[18] is associated with increased risk for atherosclerotic disease, acute myocardial infarction, and stroke.[18-23] It also increases with age and may amplify the effects of other established cardiovascular risk factors.[22] Although, the detrimental effect of plasma fibrinogen and its intermediates is not fully clear, it is thought to involve coagulation of platelets, leukocytes, free fatty acids and formation of thrombi.[24] Additionally, more recently, plasma fibrinogen has been proposed to act directly on the

endothelial wall,[21] causing a reduced ability to release endothelial-derived-relaxing-factors and decrease vasoactivity.[25] Consequently, high plasma fibrinogen contributes to an individual's acute risk of cardiovascular disease through dysregulation of the coagulation process, and to their longitudinal risk secondary to vascular dysfunction. Secondly, the relationship between plasma fibrinogen and brachial artery flow mediated dilation (BAFMD) in asymptomatic individuals had not previously been investigated.

Finally, as an exercise physiologist it was essential to perform a study involving exercise stress. Interestingly exercise training has been shown in various animal and human models to preserve or enhance the vasoreactivity of arteries over a four week training period. It is suggested the repeated stimulus of contraction-relaxation and the need to increase the delivery of blood flow to active skeletal muscle, promotes a series of vascular responses allowing increased vascular conductance and subsequent oxygen and nutrient perfusion to working skeletal muscle[26] which, in turn, may eventually lead to vessel structural changes[27].

Despite these findings the mechanisms of improvement or the time course of these changes, in humans, are not clear at this point, although are in part secondary to changes in the smooth muscle and/or endothelial vasoreactivity[28]. Accordingly, the final study in this series examined the influence of physical training on vasoreactivity. A specific aim of the study was to investigate the time course of changes in flow-mediated dilation of the brachial artery (BAFMD) in response to a 4-week localized exercise training protocol.

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CHAPTER 2. STABILITY AND REPRODUCIBILITY OF BRACHIAL ARTERY FLOW MEDIATED DILATION

2.1. Introduction

Noninvasive evaluation of flow-mediated dilation of the brachial artery has been suggested as a potential means of identifying patients with early atherosclerosis [1, 2]. The theoretical basis for the study of flow-mediated dilation is the landmark observation by Furchott and Zawadzki [3] demonstrating the obligatory role of the endothelium and endothelium-derived relaxing factors in modulating vascular tone. Additionally, Anderson et al., and Takase et al., demonstrated a close relationship between the vasoactivity of the coronary and brachial arteries [4, 5]. Consequently, the evaluation of BAFMD has been utilized as a non-invasive measure of vasoreactivity in many studies. The general consensus of these studies indicates reduced BAFMD in the presence of independent cardiovascular risk factors. For example, impaired BAFMD has been linked with cigarette smoking [6], hypertension [7, 8], hypercholesterolemia [9-11], hyperhomocystinemia [12], diabetes mellitus [13-16], plasma fibrinogen [17], and aging [8, 18].

Many of these studies utilize external ultrasound imaging devices with high-frequency linear array transducers to obtain an image of the brachial artery at rest and following a period of forearm occlusion, with the percent change in vessel diameter used as a marker of vascular function. Clearly, the appeal for using such a non-invasive, relatively painless, technique to possibly identify early/pre-clinical manifestations of vascular abnormalities is enormous. Yet despite the large number of cross-sectional studies few trials have reported the effects of behavioral or pharmacological interventions using BAFMD as an end point measure. In fact, the stability of this technique is not yet clear, and more importantly, convincing studies concerning the reproducibility of the technique are scarce.

Accordingly, the purpose of this study was to establish the reproducibility and reliability of BAFMD following five minutes of forearm occlusion. Specifically, this study examined the degree of variability of the measurement within and between test days, ultrasound testers, image readers, and subjects. Subsequently, the quantitative information was used to produce power calculations to aid in the design of simple cross-sectional, repeated measures, and prospective clinical trials with brachial artery flow-mediated dilation as an end-point measure.

2.2. Methods

Twenty-six non-smoking volunteers (38 ± 13 yr.; range: 22-57) (see table 2.1), without overt signs of disease were recruited. Individuals with acute medical conditions or active infection, on pharmacotherapy with known vascular effects (e.g. anti-inflammatory or cardiovascular medications), or those with Raynaud's phenomenon, previous arm surgery or known history of cardiovascular or kidney disease, or diabetes were excluded. Following explanation of the study, its benefits and risks, subjects signed an informed consent approved by the Pennington Biomedical Research Center Institutional Review Board.

Brachial artery assessments were obtained using high-resolution ultrasound (Toshiba Powervision SSA-380A with a 7.5-MHz linear array transducer) prior to, during, and following five minutes of forearm occlusion. Prior to the experiment subjects were instructed to fast and refrain from exercise for 12 hours and alcohol for 48 hours. Baseline ultrasound images were obtained following 15 min of supine rest. Ultrasound images were obtained in longitudinal view, approximately four cm proximal to the olecranon process, in the anterior/medial plane. With the image depth initially at 4cm, gain settings were adjusted to provide an optimal view of the anterior and posterior walls of the artery. Once settings were optimized they were kept constant throughout the experiment. All imaging was performed on the non-dominant arm with the

Table 2.1. Baseline Participant Characteristics

	Mean	SD	Range	Minimum	Maximum
Age (yr.)	37	13	35	22	57
Height (cm)	173	8	25	160	185
Weight (kg)	72	13	43	48	91
Body Mass Index (Weight/Height ²)	24	4	10	19	29
Heart Rate (beats/min)	63	12	46	34	80
Systolic Blood Pressure (mmHg)	118	13	54	104	158
Diastolic Blood Pressure (mmHg)	76	8	32	64	96
Total Cholesterol (mg/dL)	178	37	171	113	284
Low Density Lipoprotein (mg/dL)	102	33	141	48	189
High Density Lipoprotein (mg/dL)	54	14	52	27	79
Baseline Artery Diameter (mm)	3.48	0.54	1.89	2.74	4.64

subject in the supine position and forearm extended and slightly supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately one cm distal to the olecranon process, to 240mmHg for five min. Brachial artery images were recorded on super-VHS videotape for 30sec at baseline, and continuously from the final 30sec of occlusion until 5min post release. Throughout the brachial imaging procedure blood pressures were measured in the contra-lateral arm in order to account for any variation in central cardiovascular responses to the testing protocol.

All subjects were tested between the hours of 7 and 11am in an attempt to control for any diurnal variations in brachial artery vasoreactivity [19]. Additionally, blood draws were performed immediately following vessel imaging in all participants at all visits. Blood samples were examined for lipids, total protein, fibrinogen, glucose, electrolytes, viscosity factors, and homocysteine levels to ensure no significant changes occurred between visits.

Digital still images captured during diastole, as defined by the onset of the QRS complex, were subsequently analyzed (in a blinded fashion) using specialized imaging software (Media Cybernetics, Image-Pro Plus). Arterial diameters (mm) were calculated as the mean distance between the anterior and posterior wall at the vessel-blood interface.

To address intra and inter-day reproducibility 15 subjects were examined on three occasions. Intra-day assessments were separated by approximately one hour. The inter-day assessment was performed one week later. To examine technician effect on brachial artery assessment two ultrasonographers evaluated 10 participants on two separate occasions. Testers were randomly assigned to each participant. The effects of image reader bias on the brachial artery assessment, two image analysts evaluated a total of 82 images on separate occasions as

described above. Finally, to determine intra-analyst variability one analyst re-examined all 82 images, with the order of images randomized.

Statistical analyses were performed using SPSS for Windows (9.0) and SAS. Interclass correlation coefficients with confidence intervals were determined for Days, Tester, and Reader. Subsequent analysis to determine the magnitude of the variance components was performed using the SAS MIXED procedure using the REML for an unbalanced design. Tests were deemed significant at the $p < 0.05$ level.

Variance component estimates from model were then used for power calculations for three different study designs. The first of these was a simple cross-sectional approach to distinguish between groups (e.g. healthy and diseased). The second power calculation was designed for a simple pre/post longitudinal design (before and after an intervention).

2.3. Results

Brachial artery diameters, hemodynamic, and hematological data for all subjects at baseline on visit one, are shown in Table 2.1. The average baseline brachial artery diameter for the whole data set (across visits) was 3.48 ± 0.53 mm (range: 0.52 to 4.66 mm). This increased to 3.71 ± 0.57 mm (range: 0.57 to 5.04 mm) at peak dilation (within 90 sec of forearm cuff release), representing an increase of $6.58 \pm 4.15\%$ (range: -6.51 to 17.48%). The average baseline blood pressures and heart rates for the entire data set was 118 ± 12 over 75 ± 8 mmHg at 63 ± 11 beats/min, respectively. There were no significant changes in blood pressure or heart rate from baseline during the period of forearm occlusion and immediately following cuff release.

Within and between day, tester, and reader comparisons. There were no significant changes in baseline brachial artery diameters, blood pressures or heart rates between the three visits (Day 1a, Day 1b and Day 2). Furthermore, no significant changes in hematological

variables including, lipids, total protein, fibrinogen, glucose, electrolytes, and homocysteine levels were noted from Day 1 to Day 2.

The average mean percent difference in BAFMD between days ($n=15 \times 3$ days) and between testers ($n=10 \times 2$ testers) was $1.91 \pm 1.07\%$, with an ICC of 0.9188 (95%CI = 0.7255-0.9823; $p=0.0001$) and $1.40 \pm 0.97\%$, with an ICC of 0.9394 (95%CI = 0.7562-0.9850; $p=0.0001$) respectively. Additionally the mean absolute difference in BAFMD between readers was $0.21 \pm 0.014\text{mm}$, with an ICC of 0.8982 (95%CI = 0.7650-0.9615; $p=0.0001$).

One objective of this study was to determine if there were significant differences between different testers and/or readers as this may have important implications for the design of clinical trials. When these were considered as fixed effects there was a significant difference for testers ($p=0.0215$) but not readers ($p=0.5660$). The REML procedures produced estimates of the incomplete model variance components (all random effects) and these are shown in figure 2.1.

The database allowed for the fitting of a separate variance component for tester within subject by day. The estimate of this component was zero (see figure 2.1) indicating that although there may be a significant (main effect) difference between testers the difference was consistent across the period of study.

The results of the power calculations for each of the two study designs are shown in figures 2.2 and 2.3. In a cross-sectional design (i.e. smokers versus non-smokers) it is calculated that to detect a brachial artery vasoreactivity difference of 60% (two-tailed), e.g. 5% vasodilation versus 8% vasodilation, at 90% power, 23 subjects would be required. Using an interventional design the ability to detect a pre-post difference of 60% (two-tailed) in brachial artery reactivity at 90% power, 10 subjects would be required.

Figure 2.1. REML model variance components

Figure 2.2. Power Contours for a Cross-sectional Research Design

To detect a 60% (two-tailed) difference in brachial artery vasoreactivity (e.g. 5% vasodilation versus 8% vasodilation) in a cross-sectional study design (i.e. smokers versus non-smokers) at 90% power, 23 subjects would be required.

Figure 2.3. Power Contours for an Interventional (parallel) Research Design
To detect a 60% (two-tailed) difference in brachial artery vasoreactivity (e.g. 5% vasodilation versus 8% vasodilation) in a pre-post study design at 90% power, 10 subjects would be required.

2.4. Discussion

Since early work establishing blood flow-dependent increases in brachial artery dilation [4, 7, 20], several groups have used this technique as a surrogate marker of cardiovascular risk. Obviously if non-invasive evaluation of vasoreactivity is a marker of cardiovascular risk this technique could hold great clinical promise. However, prior to incorporation into clinical trials and eventually practice, it is essential the reproducibility and limitations of the technique be established.

Several laboratories, using similar techniques, have performed studies to assess the validity of the BAFMD model. Evidence of the validity of the measure has not been firmly established. However, evidence of criterion validity stems from studies linking the degree of BAFMD following occlusion to coronary vasoreactivity [21] and carotid intima-media thickness (a marker of atherosclerosis progression) [22]. Furthermore, given the plethora of studies indicating reduced vasoreactivity in individuals with established cardiovascular risk factors compared to healthy controls [6-9, 11, 13, 17], suggests the technique also has a high degree of construct validity. Unfortunately, there is a scarcity of published work to determine the predictive validity of the measure.

Relatively few studies addressed these issues, and of these few, none have controlled for factors known to influence vasoreactivity. It is therefore not surprising there is little consensus on the reproducibility of the measurement technique. For example Hardie and colleagues [14], tested 19 healthy young subjects on two separate occasions, approximately 3 months apart. Using one ultrasound technician for all scans and two readers for image analysis, they demonstrated a mean difference in BAFMD within-observers of $0.13 \pm 2.07\%$, between-observers $0.06 \pm 2.17\%$ and between-studies of $0.57 \pm 6.83\%$. Hardie et al. concluded that the reproducibility

of BAFMD measured by ultrasound was poor and likely to render the measurements inaccurate for clinical research. In fact, using the mean values provided by Hardie et al. BAFMD would have to improve by approximately 13.7% (e.g. from 3% to 16.7%), to be able to detect a change in a pre-post design. Such a change is twice the between study variation.

One of the limitations of Hardie et al. was the lack of control for variables known to influence BAFMD such as testing time of day, recent meals, caffeine or exercise. With this in mind, it is interesting that Deanfield's group [1, 23] did find adequate reproducibility in BAFMD, despite also not controlling for many of the factors known to influence vasoreactivity. In their initial study, they showed, in 21 subjects on two different days, an average difference in FMD of $2.8 \pm 2.3\%$ with a coefficient of variation of 2.3%. In 1995 they extended this work in 40 healthy subjects over four visits in a 4-month period. The fact flow-mediated dilation was calculated as the average of the values obtained by two observers may have acted to reduce measurement variability. Nevertheless, using their measures of reproducibility they estimated sample sizes for interventional trials (treatment vs placebo). Their power calculations suggested an improvement in BAFMD of 3% (for example 5% vasodilation versus 8% vasodilation) could be detected with 80% power with a sample size of approximately 25 subjects in each group [23, 24]. The findings of the present study suggest the ability to detect differences in pre and post designs using smaller sample sizes. The improvements in power calculations compared to Deanfield's group could be related to two factors; the stricter control of variables known to influence arterial vasoreactivity and possible differences in the accuracy of the measurement device.

Known hematological variables shown to influence arterial vasoreactivity include lipids [5, 11], homocysteine [12, 25], fibrinogen [17], and blood glucose [26]. Additionally, blood

pressure also has been shown to affect BAFMD. In the present study, many of these variables were monitored at each test and were not found to differ within subjects. Etsuda, et al. [19] showed a significant diurnal variation in BAFMD within subjects, demonstrating the importance of standardizing the assessment time of day. Additionally, Title et al. (2000) and Plotnick et al., (1997) have demonstrated an attenuated response in BAFMD following a glucose or fat load [26, 27]. These transient reductions lasted for up to 3 and 4 hours respectively. Clearly, this indicates the importance of the timing of meals prior to testing. The present reproducibility study is the only one to have accounted for these confounding factors by standardizing the time of day of testing (between 7 and 10am) and requiring a 12 hour fast.

In any study the precision of the measurement tool affects accuracy and limits the ability to detect a change following perturbations or interventions. The resolution of the brachial images in the current study allowed a caliper accuracy of 0.028mm. In contrast Deanfield's group were limited to a caliper accuracy of 0.065mm.

The limitations of the present study are related to the unbalanced nature of the statistical design. An a-priori decision was made to account for major variance components encountered with this technique. Consequently the study used a limited number of ultrasonographers (three) and image readers (two). In addition, between day variability was limited to only two visits scheduled, one week apart.

Considerations for the application of this technique in future studies are limited by the recency of the protocol itself. For example there are no current normative data available to indicate boundaries between "impaired" and "normal" brachial artery vasoreactivity. Furthermore, the current literature has largely focused on peak BAFMD at the 60 and 90-second post occlusion time points to identify "dysfunction". Careful study of the time course of vessel

changes following forearm occlusion is needed to arrive at a more complete understanding of the flow-mediated response. Recognizing the importance of strictly controlled conditions and the current limitations, it does appear the next step in the development of this tool is to tie in modifications of cardiovascular risk factors with changes in arterial vasoreactivity and to ultimately develop a link between arterial vasoreactivity and clinical outcomes.

These data indicate adequate stability and reproducibility of the BAFMD technique under strictly controlled conditions. Importantly, to ensure high reproducibility it is recommended study designs incorporate a single tester, or block on ultrasonographer to ensure subjects are scanned by the same individual each visit. Additionally, these results provide a series of power curves for the estimation of sample size for both cross-sectional and pre-post experimental designs using the BAFMD technique as an end-point. However, the usefulness of this technique to examine individuals on a prognostic basis is as yet, unclear.

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*CHAPTER 3. INFLUENCE OF AGE AND NORMAL PLASMA FIBRINOGEN LEVELS ON FLOW-MEDIATED DILATION IN HEALTHY ADULTS

3.1. Introduction

Elevated plasma fibrinogen [1] is associated with increased risk for atherosclerotic disease, acute myocardial infarction, and stroke [1-6]. Additionally, fibrinogen increases with age and may amplify the effects of other established cardiovascular risk factors [5]. The detrimental effect of plasma fibrinogen and its intermediates is not fully clear, but is thought to involve coagulation of platelets, leukocytes, free fatty acids and formation of thrombi [7]. Recently, plasma fibrinogen has been proposed to act directly on the endothelial wall [4], causing a reduced ability to release endothelial-derived-relaxing-factors and decrease vasoactivity. Impaired endothelium-dependent vasodilation has shown to be an early event in atherogenesis, preceding the formation of plaque [8] and occlusive vascular disease in both primate models [9] and humans [10]. Consequently, high plasma fibrinogen contributes to an individual's acute risk of cardiovascular disease through dysregulation of the coagulation process, and to their longitudinal risk secondary to vascular dysfunction. Given fibrinogen's role in vascular dysfunction we hypothesized an inverse relationship between plasma fibrinogen and brachial artery flow mediated dilation (BAFMD) in asymptomatic individuals. Accordingly, this study (1) examined the relation of plasma fibrinogen and age, and (2) evaluated the role of plasma fibrinogen on BAFMD using high-resolution ultrasonography.

3.2. Methods

Thirty non-smoking volunteers (41 ± 12 yr.; range:22-57), without overt signs of disease and normal plasma fibrinogen (283 ± 43 mg/dl; range:174-341) were recruited. Individuals with acute medical conditions or active infection, on pharmacotherapy with known vascular effects

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(e.g. anti-inflammatory or cardiovascular medications), or those with Raynaud's phenomenon, previous arm surgery or known history of cardiovascular or kidney disease, or diabetes were excluded. Following explanation of the study, its benefits and risks, subjects signed an informed consent approved by the Pennington Biomedical Research Center Institutional Review Board.

Brachial artery assessments were obtained using high-resolution ultrasound (Toshiba Powervision SSA-380A with a 7.5-MHz linear array transducer) prior to, during, and following 5min of forearm occlusion. Prior to the experiment subjects were instructed to fast and refrain from exercise for 12hr and alcohol for 48hr. Baseline ultrasound images were obtained following 15min of supine rest. Ultrasound images were obtained in longitudinal view, approximately 4cm proximal to the olecranon process, in the anterior/medial plane. With the image depth initially at 4cm, gain settings were adjusted to provide an optimal view of the anterior and posterior walls of the artery. Once settings were optimized they were kept constant throughout the experiment. All imaging was performed on the non-dominant arm with the subject in the supine position and forearm extended and slightly supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately 1cm distal to the olecranon process, to 240mmHg for 5min. Brachial artery images were recorded on super-VHS videotape for 30sec at baseline, and continuously from the final 30sec of occlusion until 5min post release. Immediately following the vessel imaging blood draws were performed. Digital still images captured during diastole, as defined by the onset of the QRS complex, were subsequently analyzed using specialized imaging software (Media Cybernetics, Image-Pro Plus). Arterial diameters (mm) were calculated as the mean distance between the anterior and posterior wall at the vessel-blood interface. Reproducibility of this technique in our laboratory yielded average mean difference in brachial artery diameter change for days, testers,

and readers of 1.91%, 1.40%, and 0.21mm respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively.

Statistical analysis was performed using SPSS for Windows (version 9.0). Group values are expressed as mean $\pm$ SD. Pearson product moment correlation was used to examine relationships between brachial artery diameter percent change (BADPC) and age and between BADPC and plasma fibrinogen. An independent sample t-test was used for comparison between participants separated by age (younger < 50yr. $\geq$ older) for plasma fibrinogen levels and BADPC. Finally, stepwise multiple regression with BADPC as the dependent variable and plasma fibrinogen, age, total cholesterol, and blood pressure as the independent variables was performed. Alpha level of $p \leq 0.05$ was required for statistical significance.

3.3. Results

Baseline data for subjects are shown in Table 3.1. Plasma fibrinogen were all within the normal range. Brachial artery diameter increased from 3.60 ± 0.71 mm at baseline to 3.82 ± 0.74 mm at peak dilation (within 90 sec of cuff release), representing a 6.08% increase (range: -3.58 to 17.48%). Correlation analysis indicated significant inverse relationships for BADPC and age $r = -0.417$ ($p = 0.02$), and for BADPC and plasma fibrinogen $r = -0.56$ ($p = 0.001$) (Figure 3.1). Comparison between the younger and older groups showed significant differences in BADPC, (younger: $7.65 \pm 3.97\%$ and older: $2.93 \pm 3.93\%$, $p = 0.005$) and plasma fibrinogen (younger: 269 ± 45 mg/dl and older: 311 ± 23 mg/dl, $p = 0.002$), but not for blood lipid or hemodynamic variables (Table 3.2).

Stepwise multiple regression analysis including plasma fibrinogen, age, total cholesterol and blood pressure revealed plasma fibrinogen as the predominant predictor for BADPC with

Table 3.1. Baseline participant characteristics

	Mean	SD	Range	Minimum	Maximum
Age (yr.)	42	12	35	22	57
Height (cm)	170	8	28	157	185
Weight (kg)	76	15	55	48	103
Heart Rate (beats/min)	63	11	50	34	84
Systolic Blood Pressure (mmHg)	120	13	54	104	158
Diastolic Blood Pressure (mmHg)	76	8	32	64	96
Total Cholesterol (mg/dL)	195	39	171	113	284
Low Density Lipoprotein (mg/dL)	114	34	141	48	189
High Density Lipoprotein (mg/dL)	56	15	64	27	91
Triglyceride (mg/dL)	116	59	275	33	308
Plasma Fibrinogen (mg/dl)	283	43	167	174	341
Baseline Artery Diameter (mm)	3.60	0.71	2.62	2.55	5.17

Table 3.2. Independent t-test comparisons between younger and older groups

	Younger (n=20)	Older (n=10)	t	p-value
Brachial Artery Diameter Change (%)	7.65±3.97	2.90±3.93	3.08	0.005*
Age (yr.)	36±11	53±3	-6.49	< 0.001*
Plasma Fibrinogen (mg/dl)	269±45	311±23	-3.33	0.002*
Peak Blood Flow Velocity (cm/sec)	178±36	169±55	0.533	0.59
Heart Rate (beat/min)	61±11	66±12	-0.98	0.336
Systolic Blood Pressure (mmHg)	117±11	127±15	-1.98	0.57
Diastolic Blood Pressure (mmHg)	76±6	76±10	0.67	0.95
Total Cholesterol (mg/dL)	189±42	208±33	-1.28	0.21
Low Density Lipoprotein (mg/dL)	112±37	118±25	-0.46	0.65
High Density Lipoprotein (mg/dL)	54±14	59±7	-0.82	0.42
Triglyceride (mg/dL)	112±62	125±52	-0.55	0.59

* = $p \leq 0.05$ between groups

Figure 3.1. Relationship between BADPC and Plasma Fibrinogen
BADPC = brachial artery diameter percent change from baseline

31% of the model variance accounted for by the equation $BADPC = 22.61 - (0.05836 * PF)$, with no other variables entered into the model.

3.4. Discussion

The unique finding of this study is the inverse relation between normal plasma fibrinogen levels and BAFMD. This finding indicates elevated plasma fibrinogen may decrease arterial responsiveness to certain vasodilatory signals such as shear stress. Given the link between impaired flow mediated dilation and cardiovascular disease [1, 5, 6, 11, 12] this suggests an additional important role for fibrinogen in maintaining chronic vascular integrity/function. In fact, multivariate analysis revealed plasma fibrinogen to be the predominant predictor for BADPC when age, total cholesterol and blood pressure were included in the model. The mechanisms of action of fibrinogen on vasoreactivity are not fully understood but may involve alterations in blood viscosity and/or chemistry causing ensuing chronic elevated stress on the artery wall with gradual depletion of endothelial derived relaxing factors. This may subsequently contribute to a loss in vasoactivity and render the vessel vulnerable to prothrombic factors and ultimately proliferation of vascular smooth muscle and possibly cardiovascular disease. The fact plasma fibrinogen is modifiable by exercise [2, 13, 14], diet [15, 16] and drug treatment warrants further study to determine if a decrease in plasma fibrinogen results in improved vascular function.

In summary, this study shows an inverse association between age-related normal plasma fibrinogen levels and brachial artery flow-mediated dilation, in 30 healthy non-smokers. Given the links between impaired flow-mediated dilation and cardiovascular disease this indicates an important role for fibrinogen in maintaining chronic vascular integrity/function, in addition to the risk for thrombus formation.

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CHAPTER 4. THE TIME-COURSE OF VASCULAR ADAPTATIONS FOLLOWING LOCALIZED SHORT-TERM EXERCISE TRAINING

4.1. Introduction

Selye's theory of stress-response-adaptation, when applied to the vascular system suggests; exercise stress leads to changes in vascular responses, followed by adaptations in the vasculature itself[1]. In this case, the stress induced by exercise results in an increased delivery of blood flow to active skeletal muscle. This repeated stimulus promotes a series of vascular responses allowing increased vascular conductance and subsequent oxygen and nutrient perfusion to working skeletal muscle[2] which, in turn, may eventually lead to vessel structural changes[3].

Increased vasoreactivity following a chronic increase in blood flow was first demonstrated by Miller and colleagues in canine femoral arteries following the opening of an arteriovenous anastomosis[4, 5]. In 1992, Lash and Bohlen, found large and intermediate sized arterioles of the spinotrapezius in aerobically trained rats (8-10 weeks treadmill exercise) exhibited greater dilation than controls, to 1-8Hz stimulation[6]. Similar responses following 8-10 weeks of exercise training have been reported in various segments of animal vasculature such as the coronary arteries,[7-10] thoracic and pulmonary arteries and arterioles,[11] the abdominal aorta[12-14] and arterioles and capillary beds of skeletal muscle[6, 15-18]. Few human studies have examined the influence of physical training on vasoreactivity. However, recent work utilizing localized resistance training programs have been shown to support an intensity independent 4-week adaptation in the forearm in the absence of central hemodynamic and sympathetic modulations[19-22]. Despite these findings the mechanisms of improvement or the time-course of these changes, in humans, are not clear. However, they appear to be, in part, secondary to changes in the vascular smooth muscle and/or the endothelium[14].

Accordingly, the purpose of this study was to examine the influence of physical training on vasoreactivity. A specific aim of the study was to investigate the time-course of changes in flow-mediated dilation of the brachial artery (BAFMD) in response to a 4-week localized exercise training protocol. It was hypothesized significant increases in peripheral conduit artery responsiveness would occur within the training period as represented by an increase in BAFMD.

4.2. Methods

Fifteen healthy, sedentary volunteers (age 26 ± 5.7 yr.) were recruited to participate in this study. Smokers, those with renal impairment or proteinuria, hepatic impairment, gout, anemia, hypercholesterolemia, hypertension, diabetes, acute medical/congestive conditions, active infection, Reynaud's phenomenon, or previous arm surgery were excluded from this study. Additionally, participants currently on pharmacotherapy with known vascular effects (e.g. anti-inflammatory or cardiovascular medications) were excluded. Each participant was required to complete an informed consent approved by the Institutional Review Boards of Pennington Biomedical Research Center and Louisiana State University.

The study was a prospective design consisting of a 4-week non-dominant arm (TR) training protocol. The protocol was localized to the forearm and the participant's dominant arm (UTR) served as the study control. Brachial artery flow-mediated dilation, defined as the absolute and percent vessel diameter change from rest, following hyperemia were the major endpoints of this study. Additionally, blood variables known to influence vasoreactivity were also examined.

Participants forearm circumferences and handgrip maximal voluntary contraction (MVC) were assessed at baseline (taken as the average of 3 consecutive trials) and on a weekly basis during training to enable the workout intensity to be adjusted appropriately. The exercise training involved

20 minutes of handgrip exercise (using a Stoelting dynamometer), at an intensity of 60% MVC and a contraction rate of 1 contraction every 4 seconds for 5 consecutive days of each week for a 4-week period[22]. Assessments of BAFMD in both the TR and UTR were made twice per week during the training period.

Blood samples were drawn before and after the training period and examined to ensure all subjects were within normal ranges, and that no significant changes occurred in variables (lipids, total protein, fibrinogen, glucose, electrolytes, and viscosity factors) known to influence BAFMD. Finally, anthropometric measures including, height, weight and 3-site skinfolds[23] along with a symptom-limited maximal arm ergometer test[24-26] were performed prior to and following the training.

The arm ergometer test consisted of the participant, in a seated position, turning an arm ergometer at a speed of 50 rpm and initial resistance of 0.5kp for 2 minutes. The resistance was then increased by 0.25kp every 2 minutes until the subject could not maintain the pedal cadence, or requested to stop.

Brachial artery assessments were obtained using high-resolution ultrasonography (with a 7.5-MHz linear array transducer) prior to, during, and following 5min of forearm occlusion. Prior to each experiment the subjects were instructed to fast and refrain from exercise for 12hr and alcohol for 48hr. Baseline ultrasound images were obtained following 15min of supine rest. Ultrasound images were obtained in longitudinal view, approximately 4cm proximal to the olecranon process, in the anterior/medial plane. With the image depth initially set at 4cm, gain settings were adjusted to provide an optimal view of the anterior and posterior walls of the artery. Once settings were optimized they were kept constant throughout the experiment. All imaging was performed with the participant in the supine position and their forearm extended and slightly

supinated. Forearm occlusion consisted of inflation of a blood pressure cuff, positioned approximately 1cm distal to the olecranon process, to 240mmHg for 5min. Brachial artery images were recorded on super-VHS videotape for 30sec at baseline, and then continuously from the final 30sec of occlusion until 5min post release. Digital still images were captured during diastole, as defined by the onset of the QRS complex, and subsequently analyzed using specialized imaging software (Media Cybernetics, Image-Pro Plus). Arterial diameters (mm) were calculated as the mean distance between the anterior and posterior wall at the vessel-blood interface. This procedure was repeated for the UTR. Reproducibility of this technique in our laboratory has previously yielded average mean differences in brachial artery diameter change for days, testers, and readers of 1.91%, 1.40%, and 0.21mm respectively, with intra-class correlation coefficients of 0.92, 0.94, and 0.90, respectively[27].

All statistical analyses were performed using SPSS for Windows (version 10.0) and SAS (version 8.1). Repeated measures t-tests were used to detect changes pre- and post-training for absolute and percent BAFMD change, forearm circumferences, MVC, maximal arm ergometer time, hemodynamic variables (SBP, DBP, HR), autonomic balance (SDNN), and hematological factors (total cholesterol, LDL, HDL, TGL, Glucose, K^+ , and Fe^+).

The differences between arms and across the training protocol for absolute and percent BAFMD were subjected to repeated measures t-tests followed by a 9 (time) * 2 (arms) analysis of variance (ANOVA) and orthogonal polynomial contrasts (linear and quadratic) used to test for trends/deviations over time. Subsequently, brachial artery responses were grouped as pre (prior to the initiation of training) and post (all measures following the initiation of training), and subjected to a 2x2 repeated-measures analysis of variance (ANOVA), blocked on subject. Alpha was set *a priori* at $p \leq 0.05$ for all statistical analyses.

4.3. Results

The initial number of participants was 16, however one was excluded for non-compliance and one subject voluntarily removed himself from the study. Participant baseline characteristics are shown in table 4.1.

All subjects completed at least 18 of 20 handgrip training sessions over the 4-week period. Repeated measures t-tests revealed forearm circumference increased by 1.24% ($p=0.001$) in the TR compared to 0.06% in the UTR ($p=0.858$) following training (see table 4.2). Additionally, MVC increased by 6.32% ($p=0.005$) in the TR compared to 4.71% ($p=0.126$) in the UTR following training (see table 4.2).

The results of repeated measures t-test for pre- and post-training measures for maximal arm ergometer time, cardiovascular hemodynamics (SBP, DBP, HR) and autonomic balance (SDNN), and hematological profiles (total cholesterol, LDL, HDL, TGL, Glucose, K^+ , and Fe^+) can be seen in table 4.3. There were no significant differences in any of the variables measured.

The TR and UTR vascular measures over the course of the training regimen are shown in tables 4.4a and 4.4b respectively. Paired sample t-tests to determine the effects of training on the TR revealed no change in brachial artery resting diameters and velocities, hyperemic blood velocity (peak rel vel), or post hyperemic diameter (peak diam). The percent ($p=0.0004$) and absolute ($p=0.007$) brachial artery changes were significantly higher following training. Additionally, a subsequent paired t-test revealed a significant difference between arms following training for the percent ($p=0.0001$) (see figure 4.1) and absolute ($p=0.0007$) brachial artery changes.

The time-course of changes in vascular reactivity following training for each participant can be seen in figure 4.2. Despite 12 of the 14 subjects showing an increase in absolute and

Table 4.1. Participant Characteristics

Variable	Mean $\pm$ SD
Age (yr.)	26 $\pm$ 5.7
Ht (cm)	177 $\pm$ 8.1
Wt (kg)	68 $\pm$ 10
BIA (%)	17 $\pm$ 4.4
BMI	22 $\pm$ 1.7
Waist/Hip	0.88 $\pm$ 0.05
SBP (mmHg)	115 $\pm$ 15
DBP (mmHg)	75 $\pm$ 6
HR (bt/min)	68 $\pm$ 10
Physical Activity Score	7.19 $\pm$ 0.8

(BIA = bio-electrical impedance; BMI = body mass index)

Table 4.2. Forearm circumference and Handgrip MVC pre and post training

Variable	Non-Dominant (trained arm) (n=14)			Dominant (untrained arm) (n=14)		
	Pre Mean $\pm$ sd	Post Mean $\pm$ sd	%change Mean $\pm$ sd	Pre Mean $\pm$ sd	Post Mean $\pm$ sd	%change Mean $\pm$ sd
MVC (Kg)	42.88 $\pm$ 8.7	45.64 $\pm$ 9.9	6.32 $\pm$ 6.1*	46.42 $\pm$ 9.7	48.54 $\pm$ 10.7	4.71 $\pm$ 10.4
Arm Circ (cm)	25.77 $\pm$ 1.7	26.09 $\pm$ 1.8	1.24 $\pm$ 1.1*	26.53 $\pm$ 1.8	26.55 $\pm$ 1.7	0.06 $\pm$ 1.2

(* = $p \leq 0.05$ as compared to pre values)

(MVC = maximal voluntary contraction; Arm Circ = forearm circumference)

Table 4.3. Hemodynamic, Autonomic, and Hematological values pre and post training

Variable	Pre Mean $\pm$ sd	Post Mean $\pm$ sd	<i>p-value</i>
Max Arm Crank (min)	7.09 $\pm$ 3.6	7.09 $\pm$ 3.8	1.0
SBP (mmHg)	115 $\pm$ 15	111 $\pm$ 8	0.269
DBP (mmHg)	75 $\pm$ 6	75 $\pm$ 5	0.887
HR (beat/min)	68 $\pm$ 10	64 $\pm$ 5	0.320
SDNN (ms) (n=7)	54.25 $\pm$ 20.38	52.13 $\pm$ 19.47	0.959
TC (mg/dl)	173.38 $\pm$ 25.9	170.15 $\pm$ 24.5	0.412
LDL (mg/dl)	104.22 $\pm$ 23.3	102.5 $\pm$ 20.3	0.609
HDL (mg/dl)	46.69 $\pm$ 8.8	45.92 $\pm$ 10.9	0.545
TGL (mg/dl)	112.93 $\pm$ 61	108.69 $\pm$ 61.7	0.808
GLU (mg/dl)	94.84 $\pm$ 5.5	94.69 $\pm$ 7.6	0.942
K ⁺ (mmol/l)	4.06 $\pm$ 0.2	4.16 $\pm$ 0.4	0.408
Fe ⁺ (ug/dl)	108.53 $\pm$ 47.6	95.08 $\pm$ 37.4	0.108

(Max Arm Crank = maximal arm crank time; SDNN = heart rate variability; TC = total cholesterol, LDL = low density lipoprotein; HDL = high density lipoprotein; TGL = triglycerides; GLU = glucose; K⁺ = potassium; Fe⁺ = iron)

Table 4.4a. Non-dominant (TR) arm brachial artery dynamics during the 4-week training regime

	Base Mean±sd	W2b Mean±sd	W3a Mean±sd	W3b Mean±sd	W4a Mean±sd	W4b Mean±sd	W5a Mean±sd	W5b Mean±sd	1 Post Mean±sd
Rest Diam (mm)	3.38 ± 0.45	3.50 ± 0.43	3.48 ± 0.46	3.52 ± 0.48	3.48 ± 0.40	3.45 ± 0.39	3.51 ± 0.48	3.52 ± 0.43	3.4 ± 0.43
Rest Vel (cm/sec)	91.41 ± 19.09	88.34 ± 23.00	98.62 ± 21.29	102.63 ± 24.11	99.96 ± 26.87	93.38 ± 20.69	92.83 ± 21.33	96.00 ± 15.47	91.39 ± 20.50
Peak Diam (mm)	3.59 ± 0.43	3.79 ± 0.47	3.77 ± 0.44	3.80 ± 0.47	3.80 ± 0.52	3.75 ± 0.39	3.80 ± 0.47	3.85 ± 0.42	3.69 ± 0.38
Peak Rel Vel (cm/sec)	150.13 ± 22.74	149.29 ± 12.18	153.30 ± 19.66	170.09 ± 34.15	163.58 ± 28.46	157.23 ± 26.21	155.35 ± 22.97	159.06 ± 19.68	156.23 ± 23.49
Absol Chg (mm)	0.20 ± 0.09	0.29 ± 0.09	0.29 ± 0.19	0.28 ± 0.04	0.31 ± 0.11	0.29 ± 0.17	0.30 ± 0.13	0.34 ± 0.14	0.27 ± 0.12
Peak Dil'n (%)	6.20 ± 3.04	8.25 ± 2.40	8.71 ± 5.71	8.47 ± 4.64	9.07 ± 3.15	8.65 ± 5.14	9.10 ± 4.08	9.99 ± 4.13	8.20 ± 4.08

(rest diam = artery diameter at rest; rest vel = blood flow velocity at rest; peak diam = artery diameter at peak dilation; peak rel vel = peak hyperemic blood velocity immediately following occlusion; absol chg = arterial diameter change from baseline to peak dilation; peak dil'n = % arterial diameter change from baseline to peak dilation)

Table 4.4b. Dominant (UTR) arm brachial artery dynamics during the 4-week training regime

	Base Mean $\pm$ sd	W2b Mean $\pm$ sd	W3a Mean $\pm$ sd	W3b Mean $\pm$ sd	W4a Mean $\pm$ sd	W4b Mean $\pm$ sd	W5a Mean $\pm$ sd	W5b Mean $\pm$ sd	1 Post Mean $\pm$ sd
Rest Diam (mm)	3.45 $\pm$ 0.39	3.49 $\pm$ 0.39	3.56 $\pm$ 0.42	3.59 $\pm$ 0.47	3.57 $\pm$ 0.44	3.51 $\pm$ 0.42	3.55 $\pm$ 0.45	3.56 $\pm$ 0.43	3.45 $\pm$ 0.37
Rest Vel (cm/sec)	83.74 $\pm$ 16.28	89.21 $\pm$ 20.31	90.29 $\pm$ 20.41	96.78 $\pm$ 19.35	95.69 $\pm$ 20.34	90.45 $\pm$ 17.38	82.53 $\pm$ 18.21	85.31 $\pm$ 14.42	79.51 $\pm$ 13.91
Peak Diam (mm)	3.61 $\pm$ 0.41	3.66 $\pm$ 0.44	3.66 $\pm$ 0.38	3.74 $\pm$ 0.45	3.73 $\pm$ 0.41	3.65 $\pm$ 0.42	3.73 $\pm$ 0.40	3.71 $\pm$ 0.38	3.63 $\pm$ 0.36
Peak Rel Vel (cm/sec)	150.97 $\pm$ $\pm$ 25.90	152.02 $\pm$ $\pm$ 16.28	156.37 $\pm$ $\pm$ 22.91	156.75 $\pm$ $\pm$ 14.73	159.29 $\pm$ $\pm$ 24.71	153.86 $\pm$ $\pm$ 20.29	146.59 $\pm$ $\pm$ 28.63	163.09 $\pm$ $\pm$ 25.35	149.23 $\pm$ $\pm$ 16.75
Absol Chg (mm)	0.16 $\pm$ 0.14	0.18 $\pm$ 0.13	0.15 $\pm$ 0.14	0.15 $\pm$ 0.12	0.15 $\pm$ 0.12	0.14 $\pm$ 0.14	0.17 $\pm$ 0.09	0.16 $\pm$ 0.17	0.18 $\pm$ 0.13
Peak Dil'n (%)	4.82 $\pm$ 4.34	4.36 $\pm$ 4.32	3.97 $\pm$ 4.7	4.13 $\pm$ 3.84	4.55 $\pm$ 3.55	4.06 $\pm$ 4.20	4.82 $\pm$ 3.31	5.20 $\pm$ 4.58	5.33 $\pm$ 3.94

(rest diam = artery diameter at rest; rest vel = blood flow velocity at rest; peak diam = artery diameter at peak dilation; peak rel vel = peak hyperemic blood velocity immediately following occlusion; absol chg = arterial diameter change from baseline to peak dilation; peak dil'n = % arterial diameter change from baseline to peak dilation)

Figure 4.1. Mean values for % change in BAFMD before and following treatment

(* = $p \leq 0.05$ as compared to pre value; † = $p \leq 0.05$ as compared to control arm post)

Figure 4.2. Non-dominant (TR) arm peak hyperemic flow-mediated dilation values for all subjects

percent BAFMD from pre to post training a 9 (time) by 2 (arm) analysis of variance (ANOVA), along with orthogonal polynomial contrast statistics failed to reveal any significant differences or patterns across the training period (see figure 4.2).

Subsequently, to provide greater statistical power for the analysis and account for inter-participant variation, the absolute and percent BAFMD means for all the post-training time points were pooled to represent a post-training measure. A 2x2 repeated-measures analysis of variance (ANOVA) for baseline and post-training (all time points pooled), blocked on subject, revealed significant differences between arms ($p=0.0157$) and an interaction effect ($p=0.0477$). Post-hoc LSD analysis results revealed no significant differences in baseline BAFMD between arms or between baseline and post training BAFMD in the UTR arm. However BAFMD in the TR arm was significantly elevated post-training (see figure 4.3).

To strengthen this finding paired t-test analysis revealed a significant increase in peak absolute ($p=0.005$) and peak percent ($p=0.015$) BAFMD following one week of training (see figures 4.4a and 4.4b). Furthermore, there were no significant differences for the TR between week 2b and the remainder of the trial.

Combined, these results indicate there was a significant increase in whole group BAFMD in the TR as compared to the UTR. Additionally, the pooled ANOVA and repeated measures t-tests suggest this change occurred by the end of the first week of training.

4.4. Discussion

This study examined the influence of physical training on vasoreactivity and specifically considered the time-course of vascular adaptations following localized short-term exercise training. The findings indicate that BAFMD was significantly elevated by approximately 62% from baseline following 4 weeks of handgrip training (6.2% to 10.0% dilation). These findings

Figure 4.3. Results of 2 * 2 ANOVA (pooled post time points)

(* = $p \leq 0.05$ as compared to pre, † = $p \leq 0.05$ as compared to control arm)

Figure 4.4a. Group mean values for peak percent BAFMD across the training regimen

(* = $p \leq 0.05$ as compared to baseline; # = $p \leq 0.1$ as compared to baseline)

(† = $p \leq 0.05$ as compared to UTR; ‡ = $p \leq 0.1$ as compared to UTR)

Figure 4.4b. Group mean values for peak absolute BAFMD across the training regimen

(* = $p \leq 0.05$ as compared to baseline; # = $p \leq 0.1$ as compared to baseline)

(† = $p \leq 0.05$ as compared to UTR; ‡ = $p \leq 0.1$ as compared to UTR)

are in agreement with previous whole body training studies reporting improvements of 77% in the brachial (2.2% dilation at baseline to 3.9% following 10 weeks of training) and 58% in the radial (8.6% dilation at baseline to 13.6% following 4 weeks of training) in healthy and heart failure populations respectively[28, 29]. These similarities are despite the fact, whole body studies do not account for adaptations in central cardiovascular control mechanisms and its influence on vasoreactivity.

A unique contribution of this study is the unilateral training design and measurement of brachial vasoreactivity in both the TR and UTR arms. Previous, studies utilizing similar designs but different assessment techniques found improvements of 30%, 23% and 20.6% in healthy young subjects forearm blood flow, following 4 weeks of handgrip training at workloads of 70%, 75% and 75% MVC respectively[19, 20, 22]. These studies utilized strain gauge plethysmography which may provide an accurate measure of blood flow at the tissue bed resistance arterioles. The present study extends the current knowledge to show a unilateral change in a large conduit vessels following training using ultrasonography. Although the comparison between whole tissue bed and single vessel adaptations are not direct ones, it is noteworthy that the directions of changes are the same.

The next logical step for this work was to demonstrate a differentiation of vasoreactivity improvements specific to the training stimulus. In 1993, Katz et al., demonstrated a regional specificity of training adaptations following 8 weeks of lower extremity aerobic training. They showed an increase in acetylcholine-mediated dilation in the trained lower extremity but not in the untrained upper extremity in normal subjects[30]. The current study demonstrated a post training unilateral improvement in vasoreactivity limited to the TR as compared to the UTR of 100.3% (10% vs 4.6%). These results are in agreement with the plethysmography work of

Sinoway et al., Green et al., and Alomari et al., who showed differences between arms of 25%, 17%, and 10%, respectively[19, 20, 22].

This aspect of the study design allows us to postulate that the improvement in vasoreactivity is a locally mediated phenomenon. The basis of this argument is two-fold; primarily, the increases in vasoreactivity were limited to only the TR and additionally, these results occurred without any alterations in central hemodynamics, autonomic balance or hematological profile. A physiological explanation for this localized improvement could involve either mechanical or chemical stimuli. For example, repetitive mechanical muscle contractions initiate rhythmical modulations in blood flow and vessel shear stresses may mediate an up regulation of the nitric oxide, and/or prostacyclin enzyme pathways[8, 31], inhibition of norepinephrine release from adrenergic nerves[32], decreased sensitivity of the α_2 -adrenergic receptor mechanism to norepinephrine[12, 13], or smooth muscle sarcoplasmic reticulum calcium unloading[33, 34]. The location of these changes is currently unclear. Several studies report increases in vasodilator mRNA and proteins at the site of the conduit vessel itself[11, 12, 14] while more recently the same laboratory suggest these changes occur in smaller distal vessels[35]. Alternatively, local accumulations of metabolites and vasoactive substances in distal tissue beds could play a role in these adaptations. In fact, previous studies have shown an increase in oxidative enzyme activity may precede the increases in vasoresponsiveness[14]. The differences in these finding could in fact be related to the duration of the training stimulus. Conduit artery protein upregulation may occur early in the exercise-adaptive process and return to normal later in the training period[35]. Interestingly, the differences in magnitude of the improvements observed between plethysmography and ultrasonography could eventually provide us with a window into the order of this vascular signaling or adaptations in response to training.

Although this idea needs further refinement it is interesting that flow-mediated dilation examinations of vascular responsiveness have consistently reported increases, whereas pharmacological approaches to vascular responsiveness and exercise training are less consistent. In fact, Joyner et al., suggests that pharmacological infusions given via the brachial artery in humans may actually reflect events at the resistance arteries rather than the conduit vessels[36]. If we consider this in conjunction with Laughlin's schema of non-uniform vasomotor responsiveness and adaptation of the arterial tree, albeit in the coronary vasculature,[3, 37] it is possible that different locations or receptors/pathways are being measured with different techniques. For example during pharmacological interventions the stimulus to dilate may precede the increase in blood flow, activating β_2 or muscarinic receptors in the smaller distal vessels, whereas flow-mediated interventions target stretch-activated potassium channels and/or some form of endothelial cytoskeleton which transduces the signal into the cell and appears to be relatively more important in the larger proximal conduit arteries[38].

The final and unique aspect of this study is the examination of the time-course of vascular adaptations following short-term exercise training. Initially, a 9*2 analysis of variance and orthogonal polynomial contrasts did not reveal any significant differences or patterns across the training period. Figure 4.2 illustrates that although 12 of the 14 participants showed increase in vasoreactivity, there is a large degree of variability in the individual plots and consequently an inability to establish a standard time-course of changes for all participants. This could be attributed to several factors. Obviously, it is possible different subjects display different time-courses of adaptation and that no 'standard curve' exists. Additionally, some participants may be hyper or hypo responders to various vascular influences which could cloud the data. Although each ultrasound scanning session was standardized as much as possible, the fact that participants

were required to attend 8 post training sessions over the course of the study lends itself to day by day variations in subject responses and variations in external influences known to effect vasoreactivity. For example time of day[39], mental stress[40-42], diet[43, 44] and the relation of food consumption to testing[45, 46] have all been shown to influence conduit artery vasoreactivity. It is also feasible that the BAFMD technique itself currently lacks the specificity/sensitivity to differentiate between individuals and is more suited to differentiating between small groups[27].

In order to examine the group time-course adaptations to the exercise training stimulus and reduce the influence of individual participant variations, the values for all the post-training time points were pooled and subjected to a 2*2 repeated measures ANOVA for arm and pre vs. post-training blocked on subject. This revealed a unilateral vasoreactivity improvement. This combined with a paired t-test analysis suggests this change occurred after only 4 days of training (see figures 4.3 and 4.4).

To the author's knowledge there is only one other published investigation involving the time-course of vasoreactivity improvements with exercise training. In 1997, Delp and associates treadmill ran rats at 30m/min on a 15% incline for 1hr/day, for 5days/week, and subsequently sacrificed them after 1 day, and 1, 2, 4, and 10 weeks. Aortic segments were then tested within 24hrs of the last exercise bout. To measure vasodilation the aortic segments were first treated with norepinephrine, which induced a similar tension in all groups. A single dose of acetylcholine was then used to induce endothelial-mediated vasodilation. The percent dilation in sedentary rats (61%) was similar to that in ring segments from animals in the 1 day, 1 week and 2 week training groups. However, the percent relaxation of the vessel rings was greater in the 4 week (72%) and 10 week (79%) trained rats[14].

The differences between Delp's findings and the current study can be attributed to many factors including species and arterial bed differences, or the fact that one study is in an intact in vitro model treated pharmacologically, whilst the other is in vivo stimulated by reactive hyperemic blood flow. Nevertheless, both studies show a time point differentiation in group responses. Interestingly, to add strength to the current findings, in 1993 Wang et al., reported an increase in nitric oxide mediated dilation response in the dog circumflex coronary artery in vivo, to both acetylcholine and hyperemia, following 7 days of treadmill exercise training[10].

Given the current understanding of the pathogenesis of atherosclerosis, which proposes that vascular dysfunction may be an initiating event[47], in conjunction with animal and human studies which consistently demonstrate vascular dysfunction in disease models, the importance of a treatment to increase vasoreactivity is tremendous. Regular exercise training may not only provide a mechanism to combat atherosclerosis by the modification of established risk factors, but may also exert a direct influence on the vascular wall itself.

In conclusion, 4-weeks of unilateral handgrip training enhanced vascular reactivity in the trained limb as compared to the control limb. The time-course of this adaptation appeared to occur following the first 4 days of training although individual participant variability reduced the statistical power of these findings. Given the relative modernity of this research area and the lack of published work addressing the questions of vascular stress adaptation it becomes obvious that the vasculature tone involves a complex interplay of many factors and additional work is required to fully differentiate the physiological mechanisms involved.

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CHAPTER 5. SUMMARY

The purpose of this final chapter is to summarize and tie together the findings and conclusions from this body of work. It must be appreciated that although this is a literal presentation of completion of requirements for a degree there are several other projects along the same theme which unfortunately did not make the cut but still exerted an influence into the design, application, and therefore the results and conclusions of these studies. These findings must also be viewed in context to the wider literature in this field, which is touched upon in Appendix A. Given the exploding nature of the volume of studies in this field this whole document can barely scratch the surface and is also outdated at the time of final printing. Taking these things into consideration, the findings of these three studies hopefully make a meaningful contribution to the field and will be significant in the direction and design of other peoples work.

The initial study (chapter 2) investigating the stability and reproducibility of brachial artery flow-mediated dilation indicated adequate stability and reproducibility of the technique under strictly controlled conditions. Importantly, to ensure high reproducibility it is recommended study designs incorporate a single tester, or block on ultrasonographer to ensure subjects are scanned by the same individual each visit. Additionally, the results provided a series of power curves for the estimation of sample size for both cross-sectional and pre-post experimental designs using the brachial artery flow-mediated dilation (BAFMD) technique as an end-point. However, the usefulness of this technique to examine individuals on a prognostic basis is as yet, still unclear.

The second study (chapter 3) investigating the influence of age and normal plasma fibrinogen levels on flow-mediated dilation in healthy adults was a cross sectional study and utilized the validated technique from study 1. This work revealed an inverse relation between

normal plasma fibrinogen levels and BAFMD. Therefore, elevated plasma fibrinogen may decrease arterial responsiveness to certain vasodilatory signals such as shear stress. Given the link between impaired flow mediated dilation and cardiovascular disease [1-5] this suggests an additional important role for fibrinogen in maintaining chronic vascular integrity/function. In fact, multivariate analysis revealed plasma fibrinogen to be the predominant predictor for BAFMD when age, total cholesterol and blood pressure were included in the model. The mechanisms of action of fibrinogen on vasoreactivity are not fully understood but may involve alterations in blood viscosity and/or chemistry causing ensuing chronic elevated stress on the artery wall with gradual depletion of endothelial derived relaxing factors. This may subsequently contribute to a loss in vasoactivity and render the vessel vulnerable to prothrombic factors and ultimately proliferation of vascular smooth muscle and possibly cardiovascular disease. The fact plasma fibrinogen is modifiable by exercise [6-8], diet [9, 10] and drug treatment warrants further study to determine if a decrease in plasma fibrinogen results in improved vascular function.

Following the identification of group difference using BAFMD it was important as an exercise physiologist to investigate the effects of an exercise intervention on the vasculature and its responses to stress. Although we know exercise has an indirect effect on vascular health by modifying hemodynamic, autonomic and hematological variables, it may also have a direct effect on the vasculature itself and if so over what period of time. Therefore the final study was a localized handgrip exercise intervention study investigating the time-course of vascular adaptations following localized short-term exercise training.

The findings of this work were unique in two ways. Initially, this was the first study to demonstrate 4-weeks of unilateral handgrip training enhanced vascular reactivity in the trained

limb as compared to the control limb. Secondly, the time-course of this adaptation appeared to occur following the first 4 days of training.

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APPENDIX A. HUMAN CIRCULATION: THE PERIPHERAL VASCULAR SYSTEM AND ITS REGULATION AND ADAPTATION TO PHYSICAL STRESS IN HEALTH AND DISEASE

A.1. Physical Characteristics of Circulation

A discussion of the circulatory system can be organized according to several different classifications that relate to the duties or tasks performed. The primary tasks of the circulatory system are the transport of oxygen and carbon dioxide. Accordingly, the most liberal discrimination deals with the exchange of gases at the lungs as a separate entity to the exchange of gases at the tissues; pulmonary and peripheral circulation respectively. Within these groupings the nature of the tissue and its specific demands for blood flow delivery and removal are reflected in the structure of the carrying vessels. For example, peripheral circulatory arteries during supine rest, are responsible for receiving approximately 80 ml of blood per second in a pulsatile manner, from the left ventricle, with pressures ranging from around 80 to 120 mmHg. They are also responsible for delivering and evenly distributing approximately 0.02-0.05ml/gram of blood to muscle tissue at pressures of around 17 mmHg. Obviously the aorta, which initially receives the blood, is very different in structure and function from the arterioles and capillaries, which finally deliver it.

“The blood flow to each tissue of the body is almost always precisely controlled in relation to the tissues needs” [1]

The structure and function of the circulatory system allow exercise increases in tissue demand of up to 30 times resting levels, to be accommodated. The heart, however, can only increase its output approximately 5 fold. Therefore, integral changes in vascular control at the vessels themselves allow for the redirection and the matching of blood demand and supply. To this end, the arteries act as pressure and direction controllers while the veins act as capacitors to

keep the heart supplied with blood, ensuring cardiac output. In this chapter the structure and function of the peripheral or systemic vasculature will be examined.

A.1.1. The Physical Anatomy of Circulation

The wall structure of almost the entire vascular system consists of three layers, an intima, a media, and an adventitia. The very thin capillaries contain only an intima. The intima is defined as consisting of a single layer of endothelial cells lining the vascular wall. In large vessels these are attached to a thin basal lamina which in turn, is connected to a sub-endothelial layer comprised of collagenous bundles, elastic fibrils, smooth muscle cells and fibroblasts [2]. The media is the middle layer of the vascular wall and consists of smooth muscle cells arranged within layers of elastic sheets and bundles of collagenous fibers. Finally, the outermost layer or adventitia consists of dense fibroelastic tissue without smooth muscle cells (except longitudinal bundles in large veins). It provides stability to the vessel and connects it to surrounding tissues. The adventitia also contains the vasa vasorum, for nutrition to the media, and nerves for vascular control. The exact composition of each of the three layers varies for different types of vessels and is generally related to the tasks each vessel performs. For a complete review of the architecture of vascular tissues please refer to [2].

A.1.1.1. Arteries

Essentially arteries can be classified as either elastic or muscular. Elastic arteries are large and initially receive blood from the heart under pressure. They act as low resistance pathways (conducting arteries) carrying blood to the medium sized muscular arteries, which flow in to even smaller arterioles, then capillaries and finally return to the heart via the venous system.

The aorta is a good example of an elastic artery. It has a diameter of approximately 15 mm and very little nervous innervation. The walls consist of both smooth muscle and elastic

laminae (40 to 60 fenestrated layers in the aorta) to enable them to resist the constant fluctuations in volume and pressure during the cardiac cycle. A major functional advantage of this is an ability to stretch during systole and “rebound” during diastole thereby helping to reduce these fluctuations and propel blood forward more “smoothly” to the delicate vessels further along the vascular tree.

Muscular arteries are immediately distal to the elastic arteries and have a diameter ranging from 10mm to 3mm, decreasing progressively away from the heart. The walls are less elastic and contain predominately smooth muscle cells. The brachial and femoral arteries are good examples of muscular arteries. Wall thicknesses of these vessels also decrease distally until they become arterioles.

Arterioles have a diameter of approximately 0.3mm to 10 μ m and a wall that often consists of just an endothelial lining and a muscular layer. Several factors can influence arterioles to change their lumen diameter including, nervous innervation, locally released metabolites and, blood-borne products.

The smallest blood vessels are capillaries with a diameter of approximately 8 to 10 μ m, which is just large enough for single erythrocytes to flow through. These vessels are responsible for the exchange of gases, nutrients at the tissue beds and are therefore, very permeable in most tissues. Essentially the walls are just endothelial cells, with clefts between them, held together on a basal lamina. The type of endothelial cells and clefts varies depending on tissue type.

Capillaries can be categorized into two types, true capillaries and metarterioles. Metarterioles act as a direct “through-way” from arterioles to venules. Surrounding this route are networks of true capillaries forming a capillary bed to better supply the adjacent tissues. True capillaries have a sphincter muscle situated at each branch point from the metarteriole,

which can be open or closed depending on the needs of the tissue. True capillaries then drain back into the metarteriole. This system allows exact matching of blood supply and demand at the local level.

A.1.1.2. Veins

Veins carry blood away from the tissues and back towards the heart. The smallest of these vessels are the post-capillary venules, which are essentially the distal end of the capillaries and generally have walls of one to two cells thick. Post capillary venules join and flow into larger venules, which in turn, join to form veins. The lumens of veins are generally larger than those of corresponding arteries yet the walls are thinner and contain less elastin. The distinction between the intima, media, and adventitia in veins is less clear than in arteries. The walls consist of a relatively weak media with, circularly arranged, plate-like bundles of smooth muscle, and an adventitia with some longitudinally arranged smooth muscle cells. Overall, the veins are less muscular and more collagenous in nature, relative to similar sized arteries [2]. The pressures endured by veins are relatively low and, in fact, they act as capacitance vessels usually containing approximately 2/3 of the body's total blood volume. To prevent backward flow of blood at such low pressures the veins possess one-way valves formed from intimal layer "cusp-like" infoldings and the muscular ability to constrict their diameter and so help ensure bloods return to the heart.

A.1.2. The Functional Anatomy of Circulation

A.1.2.1. Pressure and Volume

The anatomy of circulation must be viewed in relation to its function. The heart is simply a muscle, which acts as a gradient pump to ultimately propel whatever volume of blood it receives from the vena cava at 0mm Hg to the aorta at an average of 100 mmHg. The heart is

influenced by several external factors such as local metabolites and nervous or humoral stimulation but the basic principle remains (see figure A.1). “the heart cannot pump what it does not receive” [3].

Blood pressure is the product of cardiac output (Q) and total peripheral resistance (TPR). The parallel arrangement of organ blood supply coupled with the ability of the arteries, arterioles, and capillaries to increase or constrict their diameters, creates an effective system to control pressures and perfusions to individual tissues. The largest fall in pressure occurs at the arterioles and capillaries (see figure A.2). Pressure can also be viewed as the product of volume of blood (Q) and cross sectional area of the vessel (TPR). If we assume the volume of blood pumped per minute is unchanged then pressure becomes inversely proportional to cross sectional area of the vessels. The cross sectional area of the arteries increases from 20cm^2 in the small arteries to 40cm^2 and 2500cm^2 respectively for the arterioles and capillaries. Blood pressures have a corresponding fall from approximately 100mmHg in the large arteries to 35mmHg at the arterial end of the capillaries and only 15mmHg at the venules.

The zero pressure of returning blood at the right atria occurs primarily, due to the high capacitance of the venous system. Veins have ability to substantially increase the volume of blood they hold without a large increase in system pressure. A vein generally holds about 3 times as much blood as a corresponding artery and is approximately 8 times as distensible, that is, a given rise in pressure causes about 8 times as much extra blood to fill a vein. Therefore, as compliance is the product of distensibility and volume, a veins are approximately 24 times more compliant than corresponding arteries [1].

If we consider the effects of gravity on the upright human it becomes obvious that certain mechanisms must exist to ensure venous return. The mean systemic filling pressure in a human,

Figure A.1. The Heart as a Gradient Pump [3]

Figure A.2. Blood Pressures Across the Vascular System [3].

The pathways are shown for the example of an increase in the transmural pressure.

Symbols: framed text = established factor, unframed text = hypothetical factor

Arrows: bold arrow = well described link, normal arrow = link which needs further confirmation, dotted arrow = hypothetical link, dotted arrow with ? = unknown link

Abbreviations: AA = arachidonic acid, CYT P-450 = cytochrome P-450, K_{Ca} = calcium activated potassium channel, MP = membrane potential, SAC = stretch-activated cation channel, VOC = voltage-operated calcium channel, TK = tyrosine kinase, MLCK = myosin light chain kinase.

that is the pressure in the system after blood flow has been stopped by clamping the large blood vessels at the heart [1], is approximately 7mmHg. This is the driving pressure for venous return. Unfortunately, the force of gravity can overcome this, and in a human standing absolutely still the hydrostatic pressure or weight of the blood itself would create a pressure at the feet of approximately 90mmHg. This column of blood is prevented from forming by the action of the valve system incorporated in veins. Every time an individual constricts muscles in the leg it pushes blood to the heart and the one-way valve system prevents gravity dragging the blood back to the feet.

The circulatory system is designed to cope with a variety of situations. If pressures need to be increased, nervous stimulation can cause vasoconstriction of veins to reduce compliance, aiding venous return. Additionally, splanchnic and cutaneous vascular beds serve as large blood reservoirs, which can be mobilized to increase the volume in the circulatory system when needed. Artery beds can be dilated or constricted on a systemic or individual basis to ensure optimal perfusion of the working tissues.

The complex interplay of cardiovascular control mechanisms such as central, local, and reflex interactions render it impossible to examine this system in its entirety. While numerous studies have attempted to investigate individual control mechanisms, or manipulated one control system to examine its effects upon other variables (for a detailed review of these modulations see [4]). Unfortunately, many of these studies still fail to overcome the problems presented by redundant mechanisms or the effects across several systems, which add variability to the model (especially in disease). Additionally, many experimental designs create artificial environments in which may add outcome variability to the model. In recent years, spectral analysis and “chaos theory” have been utilized to “tease-out” system influences but even these techniques cannot

encompass the variety of possible interactive factors. It is therefore prudent to focus on a specific area within the whole circulatory system and examine direct influences upon it as of primary concern. Consequently, the focus of this literature review is the peripheral vascular system and its regulation and adaptation to physical stress in health and disease states. Details of more removed or secondary influences will be reviewed only in the context of the literature focus.

A.2. General Principles of Vascular Control

The previous section has indicated the importance of radius on the distribution of volume and pressure within the vascular tree. Poiseuille's Law best encompasses the relative influences of radius and other contributors to blood flow through arteries¹...

$$Q = \frac{\pi \Delta P r^4}{8 \eta l}$$

...whereby, Q is blood flow, ΔP is the pressure difference over the vessel length, r is the radius of the vessel, l is the vessel length, and η is the viscosity of the blood.

From this equation it is obvious radius has the greatest effect upon flow. In fact, this effect is such that a 4-fold increase in vessel radius causes a 256-fold increase in blood flow. Radius becomes an even more important contributor to flow when we consider the length of the vasculature and blood viscosity does not change transiently. It is essential therefore, to examine the factors, which impact radius.

¹ The Casson equation developed for printing inks may account for viscosity to a greater extent 5. Gow, B.S., *Circulatory correlates: vascular impedance, resistance, and capacity*, in *Handbook of Physiology: Section 2: The Cardiovascular System*, S.R. Geiger, Editor. 1980, American Physiological Society: Bethesda, Maryland. p. 353-408..

Additionally, the body must maintain adequate blood pressure and flow to supply the needs of the tissues. The best ways to do this are to manipulate cardiac output and total peripheral resistance. Since a derivative of Ohm's Law states...

$$\Delta P = Q * R....$$

...whereby, ΔP = the change in pressure across a system or vessel, Q = cardiac output or flow, and R = resistance to flow. Although the relationship between Q and R may not be linear in human vessels, it is apparent the most efficient way to increase pressure in a system or vessel is to increase the driving pressure of the fluid, increase the resistance to flow, or preferably both. In fact, a balance between these two variables is how the body regulates blood pressure and tissue perfusion.

Resistances along the cardiovascular system at rest are related to an inherent property or 'basal tone' within vascular smooth muscle.

"Basal tone may be considered as a conceptual reference point on which various vasomotor influences, both neural and hormonal, and local metabolic are expressed" [3].

That is vasoconstrictors can be used to increase basal tone and resistance to flow for the whole system or at a local level. Likewise vasodilators can be used to reduce vascular resistance.

This section will outline major systemic mechanisms underlying Poiseuille's Law and central blood flow/pressure regulation. The major focus will be devoted to local circulatory regulation, with an emphasis on the contributions of the intima, media and, adventitia to flow regulation including, myogenesis, sympathetic stimulation or inhibition, flow, and humoral influences including, local metabolites, endothelin, vasopressin, bradykinin, prostaglandins, and endothelium-derived nitric oxide [6, 7].

A.2.1. Systemic Blood Pressure Control

The most influential control mechanism for short-term blood pressure regulation is the baroreflex or pressoreceptor (for a comprehensive review see [8]). These consist of stretch receptors in most of the large arteries of the thorax and neck (especially the aortic arch and carotid sinus), which respond to changes in pressure and rate of change in pressures between 60 and 180 mmHg for the carotids and 90 and 210 mmHg in the aorta. Increases or increasing pressures cause nerve transmission to the tractus solitarius in the medulla of the brainstem and subsequent inhibition of the vasoconstrictor center with concomitant excitation of the vagal center. The resulting cardiovascular responses are vasodilation of the peripheral arterioles and veins with decreased heart rate and force of contraction. Subsequently, blood pressure falls. Conversely, a fall in arterial pressure results in withdrawal of baroreceptor signals to the medulla, an increase in cardiopulmonary receptor stimulation and the reverse effects. The importance of the peripheral arteries vasoconstrictor response is most evident in arterial pressure decreases subject to conditions which limit ventricular filling pressures (e.g. upright posture, heat stress) and prevent a rise in cardiac output [3].

Medium-term (hours) and long-term (days or weeks) blood pressure control is predominantly controlled by a process of adjusting the rate of water intake and the rate of solute-free water excretion by the kidney and by vasoconstriction of the vasculature (for a complete review see [9]). Vasopressin is the major hormone involved in medium-term water retention during hypovolemia/hypotension or during an increase in plasma osmolarity. During these conditions the hypothalamic-neurohypophyseal system stimulates a release of vasopressin from the posterior pituitary gland. Vasopressin acts to increase the permeability to water of the kidney collecting ducts via V_2 receptors and the adenylate cyclase/cyclicAMP pathway. To a lesser

extent vasopressin stimulates a pressor response consisting of vascular smooth muscle (and other tissue) contraction via V1 receptors and G-protein/arachidonic acid pathway.

Chronic decreases in pressure or flow to the kidneys create a cascade of events, known as the renin-angiotensin system, designed to restore normal perfusion. This process starts with the release of the kidney hormone renin. Renin is synthesized, stored and secreted into the renal arterial circulation by the juxtaglomerular cells in the afferent arterioles as they enter the glomeruli. Renin has a half-life of approximately 15-min. Specifically, renin can be stimulated via three mechanisms...

(i) Macula densa pathway.

The macula densa is situated adjacent to the juxtaglomerular cells and is composed of specialized columnar epithelial cells located in the wall of that portion of the cortical thick ascending limb that passes between the afferent and efferent arterioles. A decrease in NaCl reabsorption by the macula densa stimulates renin release (using adenosine), conversely an increase causes the opposite effect of renin inhibition (via prostaglandins) [10].

(ii) Intrarenal baroreceptor pathway

Increases and decreases in preglomerular vessel blood pressures inhibit and stimulate renin (via prostaglandin) release respectively.

(iii) β -adrenergic receptor pathway

This mechanism is mediated by the release of norepinephrine from postganglionic sympathetic nerve terminals. The activation of the juxtaglomerular cells β -adrenergic receptors enhances renin release.

An increase in renin causes an increase in angiotensin II, which, in turn, inhibits renin release by effecting subtype 1 receptors on the juxtaglomerular cells in a short-loop negative

feedback system. Additionally, there is a long-term mechanism of negative feedback via the angiotensin II effect of systemic blood pressure elevation and thereby, reducing sympathetic β -adrenergic juxtaglomerular cell stimulation.

The substrate for renin is angiotensinogen, which is continuously produced, primarily in the liver but also by areas of the central nervous system and the kidney. Glucocorticoids, thyroid hormone and angiotensin II stimulate angiotensinogen production. Renin cleaves angiotensinogen between amino acids 10 and 11 (the leucine-valine bond), to create angiotensin I, which is a mild vasoconstrictor. It is usually quickly converted to angiotensin II by angiotensin-converting-enzyme (ACE) which is bound to the endothelial cells in the vascular system. Angiotensin III is also produced from angiotensin I, via a minor pathway. ACE cleaves dipeptide units from substrates with amino acid sequences in a non-specific manner.

Angiotensin II and III have vasoconstrictor effects on the peripheral vasculature, but angiotensin II is considerably more potent. In fact, angiotensin II is considered to be approximately 40 times more potent than norepinephrine in this regard [10]. Angiotensin II activates AT_1 receptors on the vascular wall and has two major effector pathways...

- (i) Smooth muscle constriction occurs via guanine nucleotide-binding regulatory (G) proteins, which facilitate activation of phospholipases C- β and eventually lead to an opening of sarcoplasmic reticular stores and calcium channels and an increase in intra-cellular calcium levels. Calmodulin sequesters calcium ions and forms calmodulin-calcium complexes that subsequently activate ATPase-kinases and muscle contraction occurs.
- (ii) Phospholipase A_2 is stimulated at the receptor and it metabolizes phosphatidylcholine to arachidonic acid. Arachidonic acid can then modify cellular responses by conversion to prostaglandin and thromboxane A_2 or by activation of its own receptors. AT_1 receptors may

also stimulate cell growth, especially cardiac and vascular muscle cells, via the MAP kinase pathway and increased expression of proto-oncogenes, particularly *c-fos* and *c-jun*.

The major effects of angiotensin II are the rapid pressor response via altered peripheral resistance, the slow pressor response via altered renal function, and the long term vascular and cardiac hypertrophy and remodeling.

(i) The rapid pressor response

“When a single moderate dose of angiotensin II is injected intravenously, systemic blood pressure begins to rise within seconds, rapidly reaches maximum, and returns to normal within minutes” [10].

The main contributor to this response is an elevation of total peripheral resistance by vascular smooth muscle AT_1 receptor stimulation and contraction. Angiotensin II may also enhance peripheral noradrenergic neurotransmission by augmenting norepinephrine release and inhibiting its uptake at sympathetic nerve terminals and by enhancing the vascular response to norepinephrine. Interestingly the angiotensin II molecule itself may have some neurotransmitter or modulator effects on the central nervous centers.

(ii) The slow pressor response

The slow pressor response is mediated predominantly by an alteration of renal function via increased retention of Na^+ and water coupled with an increase excretion of K^+ . This occurs through several mechanisms including a direct angiotensin II effect on the proximal tubule, a modification of glomerular flow rate by vasoconstriction of renal arterioles, and stimulation of the adrenal cortex to release aldosterone. The overall effect of angiotensin II on the kidney is to move the pressure natriuresis curve to the right.

(iii) The vascular and cardiac remodeling response

Angiotensin II has been shown to cause hypertrophy and remodeling of cardiovascular tissues including; increased blood vessel wall-to-lumen ratios, vessel intimal thickening and both eccentric and concentric cardiac hypertrophy. This is attributed to the hypertension and the increased proto-oncogene expression.

A.2.2. The Adventitia: - Neural Control

The sympathetic vasomotor system is the most pervasive influence in neural regulation of circulation. It carries a tremendous number of vasoconstrictor fibers and only a few vasodilator fibers. Their anatomy is such that they leave the spinal cord through all the thoracic and the first two lumbar spinal nerves. This system innervates the entire vascular arterial tree down to the arterioles. Capillaries, precapillary sphincters, and metarterioles are not innervated. The sympathetic postganglionic fibers are usually nonmyelinated and form two plexuses in the tunica adventitia. The outer or primary plexus is usually present in the middle or outer third of the adventitia and its fibers ramify and terminate more distally in the vascular bed. The inner or terminal plexus is typically restricted to the adventitio-medial junction, and transmitter release here leads to changes in vascular smooth muscle tone [11]. The sympathetic neurons are predominately adrenergic, releasing norepinephrine, which binds at lipoprotein α receptors on the cell membrane, where the vasoconstrictor response is initiated [3].

The neuroeffector junction is a dynamic balance between norepinephrine release and uptake or diffusion, and can be influenced by other vasoactive substances such as angiotensin II, nitric oxide, and local metabolites. The synthesis of norepinephrine from tyrosine is a complex process involving several intermediates (see [11, 12]). A vast majority (approximately 80%) of the norepinephrine released is reincorporated into the releasing nerve terminal via α_2 receptors,

and a small amount spills over into the circulation. It must be noted here, in the adrenal medulla there are two distinct catecholamine containing cell types: one with norepinephrine (as in neuroeffector junctions) and one with primarily epinephrine, synthesized by phenylethanolamine-N-methyltransferase from norepinephrine. Epinephrine (0.2 $\mu\text{g/kg/min}$) and norepinephrine (0.05 $\mu\text{g/kg/min}$) are released by the adrenal medulla directly into the blood stream and contribute significantly to basal vascular tone. Interestingly, epinephrine and norepinephrine have different effects at the vasculature compared with the heart. Epinephrine affects both α and β receptors similarly whereas, norepinephrine has a much greater effect on α receptors.

The parasympathetic system releases acetylcholine via the vagus nerve and in general has the opposite effect to sympathetic stimulation. For example, parasympathetic discharge at the myocardium causes a decrease in heart rate and force of contraction. However, the parasympathetic system has little or no effect on peripheral resistance at the vasculature. Vanhoutte (1980), suggested parasympathetic discharge close to the adrenergic nerve ending may cause a hyperpolarization effect and create a resistance to activation and a subsequent dampened norepinephrine release [13].

Having examined the vasoconstriction response it is necessary to detail the theories of vasodilation. The existence of sympathetic cholinergic vasodilators is established in muscle tissues of some species. Their effect is transient at best and an understanding of the rationale for these mechanisms is unclear. In human skeletal muscle the evidence for cholinergic vasodilators is not strong [3]. Other neurogenic vasodilator mechanisms have been proposed but in humans peripheral vascular resistance and blood pressure are maintained by alterations in basal tone and sympathetic vasoconstrictor withdrawal.

Further strata in the complexity and co-ordination of vascular behavior are the varying degrees of responsiveness of select tissues to sympathetic stimulation and the degree or manner of discharge of the system. Under certain circumstances such as fright or pain a mass sympathetic discharge occurs. In exercise or heat regulation specific areas can be constricted while others undergo dilation to produce optimal blood flow to the working tissues or skin [14]. Additionally, there are significant regional variations in the responses of arterioles to a similar sympathetic stimulation and the density of α -adrenergic innervation differs greatly. Arterioles supplying skin, skeletal muscle, splanchnic organs, kidneys, and adipose tissue are richly innervated. Innervation in the veins is variable from very low deep in limb tissue to very high in the splanchnic and cutaneous tissues [3]. Additionally, the receptor themselves may be slightly varied in different locations. Finally, the norepinephrine release-reuptake balance may vary with location.

A.2.3. The Media: - Myogenesis (The Bayliss Effect)

Autoregulation of blood flow is the tendency for flow to remain the same despite changes in arterial pressure [15]. This behavior, termed the myogenic response, is inherent to smooth muscle and independent of neural, metabolic, and hormonal influences [16]. Interestingly, there appears to be an inverse relationship between vessel size and myogenic responsiveness [17], although this response may also differ in different types of tissues [18]. It is most pronounced in the arterioles (for a comprehensive review of myogenesis please refer to [15, 17]) and may act as a protective mechanism to avoid over-perfusion of the capillary beds. This effect was first noticed in 1902 by William Maddock Bayliss in dog the hind limbs following release of brief aortic occlusions [19]. Folkow's work with denervated preparations established myogenesis as a pressure dependent, non-neural mechanism [20]. Currently, the stimulus for the myogenesis is

unclear but is believed to be a response to tension changes on the vessel wall and/or to stretch on the myofibrils. Part of the reasons for this uncertainty are the differing magnitudes, time courses and directions of stimuli in isometric contractions of vascular rings/strips compared with isobaric contractions of cannulated arterioles and arteries [21].

The wall tension theory has recently gained support through mechanistic studies demonstrating alterations to agonist sensitivity only occurs with changes in wall tension. Additionally, events implicated in myogenic signal transduction such as intracellular calcium concentration and myosin light chain phosphorylation are significantly correlated with vessel wall tension, not vessel diameter [22]. Additionally, physics supports the wall tension theory. The law of La Place dictates wall tension (T) is a function of transmural pressure across the vessel wall (Pt) and radius (r).

$$T = P_t * r$$

Therefore, if wall tension increases due to a rise in pressure inside the vessel, a reduction in radius can counteract this increased wall tension and create a homeostatic feedback loop.

The actual sensor of pressure is as yet unclear. Stretch activated cation channels, voltage dependent calcium channels, and the extracellular matrix have all been proposed as mechanisms [18], but conclusive evidence is still lacking. The structural component of the vessel involved in myogenesis appears to be the smooth muscle itself. Removal of the vascular endothelium has led to contrasting results mainly between physical removal, which led to an altered response, and chemical removal, which did not. Further analysis of these studies leads to the conclusion that myogenesis in the brain may be more endothelium dependent, as may some of the larger conduit arteries which, also endure greater flow velocities [23]. Interestingly, removal of tonic nervous influence by chemical blockade caused no alteration of myogenic response. Consequently,

myogenesis is believed to be predominately a reaction of the smooth muscle cells in the vessel walls of small arteries and arterioles, independent of either nerves or endothelium.

An increase in blood pressure causes an increase in vessel wall tension and in turn, initiates depolarization of the smooth muscle cell membrane and a cascade of second messenger pathways resulting in contraction (figure A.3). From figure A.3 it is established that an increase in calcium in the smooth muscle cytosol accompanies myosin light chain phosphorylation. The concentration of calcium necessary to initiate contraction is unclear. It may be possible that auxiliary pathways alter calcium the sensitivity of the smooth muscle contractile apparatus.

Unfortunately, in vivo external influences pervade myogenesis. For example, blood flow is pulsatile not static. Additionally, nervous stimulation of the smooth muscle tissue may enhance the myogenic response, which is in direct contrast to some endothelially released relaxing factors or local tissue metabolites, which may antagonize the response. It is therefore difficult to generalize from in vitro responses to stimuli to intact animal autoregulation.

In conclusion, the myogenic response is a reaction to a change in wall tension and is demonstrated in a variety of tissue beds to varying degrees. It involves smooth muscle cell depolarization and an influx of calcium ions. Furthermore, it appears not be dependent upon the endothelium or on nervous stimulation yet in-vivo can be modified by both of these.

A.2.4. The Intima : - Endothelial Control

The unique anatomical position of the endothelium, at the interface between the lumen and media of blood vessels, lends itself to multiple roles in the maintenance of vascular tone [24]. The importance of the vascular endothelium to arterial vasomotion was first demonstrated by Furchgott and Zawadski, (1980), whereby, the presence of a healthy endothelium was essential for normal vasodilation [25]. Despite being only a single layer of cells it can “detect”

Figure A.3. A Model of the Main Pathways Suggested to be Involved in the Myogenic Response

The pathways are shown for the example of an increase in the transmural pressure.

Symbols: framed text = established factor, unframed text = hypothetical factor

Arrows: bold arrow = well described link, normal arrow = link which needs further confirmation, dotted arrow = hypothetical link, dotted arrow with ? = unknown link

Abbreviations: AA = arachidonic acid, CYT P-450 = cytochrome P-450, K_{ca} = calcium activated potassium channel, MP = membrane potential, SAC = stretch-activated cation channel, VOC = voltage-operated calcium channel, TK = tyrosine kinase, MLCK = myosin light chain kinase [15].

changes in hemodynamic forces, or blood-borne signals, by membrane receptor mechanisms and respond to physical and chemical stimuli by synthesis or release of a variety of vasoactive and thromboregulatory molecules and growth factors [26]. These include endothelial-derived relaxing factor/nitric oxide [25, 27], endothelial-derived hyperpolarizing factor [28], prostacyclin [29-31], bradykinin, thromboxane, endothelin [32], and tissue-type plasminogen activator [33, 34].

Endothelial structure and function have been established through in vitro culture studies and through in-vivo animal [35] and human studies [36]. These findings have led to the development of cascade models for stimulation and reaction of the endothelium (Figure A.4). Bradykinin, substance P, serotonin, adenosine diphosphate and adenosine triphosphate all depend upon the presence of the endothelium for their relaxant action [37]. Two general classes of stimuli elicit an endothelial-dependent response. The first of these is pharmacological stimuli such as norepinephrine, acetylcholine, and bradykinin via specific endothelial cell receptors. NE stimulates α_2 receptors, bradykinin stimulates β_2 receptors, and acetylcholine (although its humoral levels would be sufficiently high enough under normal conditions) stimulates muscarinic receptors. Specific G proteins activate phospholipase C (PLC) which leads to the hydrolysis of phosphatidyl inositol bisphosphate into two intracellular second messengers: inositol triphosphate (IP_3) and diacylglycerol (DAG). Consequently IP_3 triggers calcium release from intracellular stores [38].

Additionally, flow induced shear stress is believed to increase intracellular calcium concentration via stretch activated potassium channels and/or some form of endothelial cell cytoskeleton which transduces the signal into the cell [16]. Shear stresses range between 10 and 70 dyne/cm² at different points in the arterial vascular network [40]. Changes in these shears

Figure A.4. A simplified model of receptor-signal transduction processes in endothelial cells leading to the release of putative endothelium-derived prostacyclin (PGI₂), endothelium-derived hyperpolarizing factors (EDHF), and endothelium-derived relaxing factor, nitric oxide (EDRF-NO). Vasoactive substances (hormone) such as: norepinephrine, UK14304, acetylcholine, bradykinin, and substance-P, bind to their specific endothelial cell membrane receptor that is coupled by a G protein to phospholipase C (PLC). Activation of PLC leads to hydrolysis of phosphatidyl inositol biphosphate producing two intracellular second messengers: inositol trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ signals calcium release from intracellular stores. Increased intracellular free calcium ([Ca²⁺]_i) activates three processes: 1) phospholipase A₂ to release arachidonic acid (AA) which is converted to prostacyclin by cyclooxygenase; 2) nitric oxide synthase (NO synthase) to produce EDRF-NO; and 3) release of unidentified EDHFs by as yet to be established pathways (?). As illustrated, flow-induced vasodilation results from increased shear stress which induces increased [Ca²⁺]_i via stretch activated channels (SAC) or G-protein mediated mechanisms. PGI₂ is believed to signal relaxation of vascular smooth muscle by stimulating adenylate cyclase which produces cAMP (adenosine 3'-5'-monophosphate) from ATP (adenosine triphosphate). EDRF-NO is believed to signal relaxation in vascular smooth muscle by stimulating guanylate cyclase which produces cGMP (guanosine 3'-5'-cyclic monophosphate) from GTP (guanosine 5'-triphosphate). As illustrated by the '?'s, the process whereby EDHF is produced and mechanism by which EDHF causes hyperpolarization are not established [39].

have been shown to influence vessel diameter responses. Higher shear stress caused from higher flow or viscosity leads to an increase in internal vessel radius. Conversely lower shear stress leads to decreased internal vessel radius. It is suggested that the response of endothelial mediated compensatory changes are to maintain shear stresses in the range of approximately 15 to 20 dyne/cm² [41, 42]. Rat studies in which shear stress is acutely altered by aortocaval shunt results in upregulation of cGMP [43] along with NOS messenger mRNA. These changes are accompanied by vessel structural expansion and could be blocked by L-NMMA (see below).

Intracellular calcium concentration is believed to be the key mediator for vasodilation by activation of three mechanisms; NO, prostaglandin and endothelial-derived hyperpolarizing factor.

(i) NO –GTP to cGMP

NO is the most discovered and influential contributor to vascular tone under normal conditions. There are several isoforms of NO released endothelially. Constitutional NO contributes to vascular basal tone by inhibiting contraction of the underlying smooth muscle, whereas inducible NO is released in response to stimuli. NOS is the enzyme responsible for NO production and also exists in both constitutive (basal) and inducible isoforms. NOS converts the substrate L-arginine to L-citrulline and NO in the endothelial cells. This can be blocked by arginine analogues, of which N^G-monomethyl-L-arginine (L-NMMA) is the most widely utilized [44]. The vasodilator activity of NO occurs in the vascular smooth muscle, due to its interaction with the iron atom of heme in guanylate cyclase,[45] causing its activation as the enzyme to produce cyclic guanosine 3'-5'-monophosphate (cGMP) from guanosine 5'-triphosphate (GTP) [38]. cGMP activates several protein kinases causing calcium extrusion from the cell, increased uptake by the sarcoplasmic reticulum and subsequent dilation. NO also displays some hyperpolarizing

characteristics. It is capable of activating calcium-dependent potassium channels and ATP-dependent potassium channels indirectly, by increasing intracellular cGMP.

(ii) PGI – ATP TO cAMP

The increased endothelial intra-cellular calcium concentration activates phospholipase A₂ to release arachidonic acid (AA), which is converted by the enzyme cyclooxygenase to prostacyclin (PGI₂) [46]. This pathway can be inhibited by cyclooxygenase blockade. PGI₂ itself has antithrombic and antiplatelet properties. Its vasodilatory capacity in vascular smooth muscle is by the activation of the enzyme adenylate cyclase to convert adenosine triphosphate (ATP) to adenosine 3'-5'-monophosphate (cAMP), with subsequent activation of protein kinases. PGI₂ may also demonstrate some hyperpolarizing characteristics by activation of smooth muscle cell ATP-dependent channels through the secondary rise in cAMP after binding to the corresponding cell surface receptor.

(iii) EDHF

The hyperpolarizing characteristics of both NO and PGI₂ have created difficulties in identifying the mediator for EDHF. In fact, its relaxing effect can only be revealed following combined blockade of NO and PGI₂ [28, 47]. Relaxation attributed to this factor occurs secondary to smooth muscle cell hyperpolarization and is blocked by membrane depolarization [28]. In most vascular beds calcium dependent potassium channel inhibitors inhibit this response. A cytochrome P450-derived arachidonic acid metabolite has been suggested as EDHF for human coronary circulation [48], but it is possible different factors are responsible in different vascular beds.

EDHF may only become a significant contributor to vasodilation when NO and PGI₂ are inhibited in arteriosclerotic, hypercholesterolemic, or ischemic conditions, possibly as a

compensatory or reserve mechanism [47]. This is supported by EDHF's apparent insensitivity to superoxide anions.

A.2.5. Metabolic Dilation

The relative importance of metabolic control increases with decreasing vessel size, from large capacitance vessels to smaller precapillary resistance vessels [39]. Vasodilator substances (e.g. adenosine, adenine nucleotides, carbon dioxide, PO_2 , potassium, phosphate ions, lactate, Krebs cycle intermediates, and various polypeptides), which, are released from active muscle fibers, diffuse through the interstitial space to act on the smooth muscle cells of arterioles [3, 49]. Several theories have postulated many different substances be “the” controlling vasodilator. This seems unlikely because, individually these metabolites have modest effects, but when combined they elicit a greater vasodilatory response. In fact, in 1972, Lundvall, hypothesized that the total osmolarity of all particles released by the active tissue was the cause of vasodilation [50]. In fact, total osmolarity of venous blood increases in proportion to organ oxygen consumption and blood flow. Additionally, in resting tissues blood flow can be raised to exercise levels by infusions, which appropriately increase osmolarity [3]. Unfortunately, this matching effect only lasts for a few minutes and then becomes complicated by increased blood flow to the tissue that reduces metabolite interstitial concentrations. It is interesting to note that distal vasodilation causes a reduction in distal total peripheral resistance and thereby encourages increased flow in the proximal conduit arteries and consequent endothelial flow-mediated dilation, which may in turn create an increase in blood flow to those distal tissue beds [51].

Arteriolar PO_2 has been investigated as a vasodilator influence. It is logical that as smooth muscle needs O_2 for contraction, reduced levels will initiate relaxation. Unfortunately, although low venous PO_2 is associated with vasodilation the relationship does not track each

other except in conditions of extreme hypoxia [49]. Arterial PO₂ must fall below 40 to 50 mmHg before flow is affected, thus the sensitivity required for oxygen tension to regulate flow appears not to exist [3]. It is possible CO₂ and hydrogen ion fluctuations have a direct effect on vascular smooth muscle and flow over a narrow range. This relationship is established in cerebral vessels but currently, not in other tissue beds.

It is possible, with recent development in the understanding of endothelial function, that hypoxic status could cause a release of local vasodilators such as adenosine derivatives and prostaglandins [52]. Adenosine itself, unlike its nucleotides, crosses cell membranes easily and its interstitial concentration should give a relatively good parallel of cellular metabolism. Unfortunately, adenosine is an unsuitable candidate for vasodilatory regulator due to the low levels in the venous blood and the rapidity of its conversion to inactive compounds.

Another local influence on vasoregulation is temperature. Cutaneous vessels have unique responses to temperature changes. For example, cooling creates greater sensitivity to sympathetic stimulation [13]. In contrast deeper vessels lose some myogenic activity and are less sensitive to vasoconstrictor stimuli. Warming tends to decrease adrenergic sensitivity and thereby reduce vasoconstriction

In conclusion, the difficulty in acquiring accurate interstitial concentration measures and because different influences may effect vasoactivity at different times in the course of dilation or exercise. A further complication is the possibility of redundant systems capable of regulating blood flow when primary mechanisms are inhibited. It is interesting to consider these different influences on vasocontrol acting in parallel to each other homogeneously throughout the vascular network. However, this does not appear to be the case, at least in the coronary arterial tree (Figure A.5) [38, 53]. In 1995, Jones et al. proposed the theory of interconnected vascular

Figure A.5. Schematic illustrating the relationship between the relative responsiveness of segments of the coronary arterial tree to vascular control systems and the diameter of each arterial segment. The *top panel* illustrates the coronary arterial tree and the *second panel* reflects changes in diameter observed on progression from the conduit coronary arteries down to the resistance arteries and arterioles. The last four panels illustrate the relative responsiveness of the segments of the arterial tree respectively to: myogenic reactivity, flow-induced vasodilation, metabolic vasodilator signals, and sympathoamines [39].

“micro-domains” [54]. Each micro-domain is conceived to be primarily under the control of specific regulatory mechanisms, for example myogenesis along with metabolic control appear to be of greatest importance in small resistance arteries and very small arteries respectively (Figure A.5). Conversely, the larger conduit arteries are more responsive to flow and sympathetic stimulation. Overall the micro-domains of the coronary tree are then proposed to be integrated by interactions between metabolic, myogenic, and flow dependent control processes [54], with different ones serving as the dominant influence and others as redundant systems in different micro-domains and presumably under different physiological conditions.

A.3. Assessment Methods for Peripheral Circulation

There are several recognized methods of assessing peripheral circulation. Some methods are more applicable for basic and animal research while others are regularly employed in clinical assessments. Several techniques have been developed to enable blood flow estimates to be made of the arterial inflow, venous outflow, or local blood flow within the muscle [55]. Regional techniques include electromagnetic flow meters, plethysmography, indicator methods (thermodilution and indocyanine green dye infusion), Doppler Ultrasound, and magnetic resonance imaging. Local blood flow techniques include Xenon-133 clearance, microdialysis, near infrared spectroscopy, positron emission tomography. As some techniques continue to evolve they may be used for both regional and local flow detection (near infrared spectroscopy coupled with indocyanine green dye). This review will describe several of these techniques and discuss their strengths, limitations and applications. Particular emphasis will be placed on the popular non-invasive techniques of venous occlusion plethysmography and the recent

development of Doppler Ultrasonography. Additionally, as it is most pertinent to the author's field of study Doppler ultrasonography will form the major concentration of this section.

A.3.1. Regional Blood Flow Measures

A.3.1.1. Electromagnetic Flow Meters

The principle behind electromagnetic flow meters is based on the fact that blood is a conductive liquid. Consequently, any induction of voltage change will be proportional to the blood flow through the vessel being tested [56, 57]. Therefore...

$$\text{electromotive force} = B * L * u$$

...whereby, B represents the magnetic flux density (Torr), L represents the distance between the electrodes (m), and u is the instantaneous blood velocity (m/s) [55]. Blood flow can then be calculated from the mean blood flow velocity and the cross-sectional area of the vessel. The flow probe is mounted around a particular blood vessel in an invasive procedure. The probe itself generates a strong electromagnetic field to detect voltage deflections. Advantages of this system include the ability to calculate instantaneous volumetric pulsatile flow. Additionally the equipment is relatively inexpensive to purchase and operating costs are low. Unfortunately, this procedure is too invasive for use as a standard method for measures in healthy human subjects and is therefore mainly limited to animal research.

A.3.1.2. Plethysmography

Plethysmographs record a biological change in volume or other dimension (such as light reflectance) in a portion of the body associated with cardiac contractions, respiration, or in response to pneumatic venous occlusion [58]. A variety of transducers are available including air, photocell, and the most popular strain gauge. These allow this technique to be employed in the detection of peripheral-vascular disease, venous disease, and carotid artery disease.

Complete coverage of all aspects of plethysmography is beyond the scope of this review. Venous blood flow detection utilizing strain gauge venous occlusion plethysmography will be the focus here.

“For measuring total limb blood flow, the most useful and almost certainly the most reliable method, in skilled hands, is venous occlusion plethysmography.”[59]

This technique involves stopping venous return for a few seconds without disturbing arterial inflow. Continuous recordings of whole limb (or isolated segment thereof) volume changes are made. The initial rate of swelling is the apparent rate of arterial inflow (assumed to be the same as before venous occlusion). The arm should be at or above the level of the heart and tilted to ensure venous drainage. Interestingly, application of a wrist cuff (one minute prior to venous occlusion was shown to provide lower forearm flows than in when the hand was not occluded [60]. Presumably, when the hand is not occluded, some of the arterial inflow to the hand may accumulate in the veins of the forearm.

The mathematical principle behind strain gauge plethysmography is that the percentage change in volume of a cylinder of fixed length is approximate to twice the percent change in its circumference. This approximation also applies to sub-cylindrical objects of non-uniform cross-section, if the changes are in uniform proportion at all levels along the fixed axial length. The linear relationship between changes in volume and circumference may therefore be expected to apply to a segment of human limb [59]. Criticisms of this technique come from the derived volume change rather than a measured one as with the water plethysmograph. Direct comparisons show that values obtained by both techniques, under controlled conditions are similar [61], although others have observed the water technique recorded flows approximately 9% higher than the strain gauge technique [62]. These differences may be related to Hiatt and colleagues findings that inflation of a venous occlusion cuff during strain gauge measurements

reduced radial arterial diameter and blood flow velocity, observed using Doppler ultrasonography [63]. Nevertheless, strain gauge plethysmography does appear to be reproducible for serial measurements following reactive hyperemia [64-66], and following 5 minutes of forearm occlusion coupled with handgrip exercise [64, 66]. Interestingly, reproducibility of resting blood flows is less clear. Mahankali et al. showed only correlation coefficients of approximately $r=0.55$ for resting forearm flows between and with days [64].

The benefits of strain gauge plethysmography over other techniques is the ability to measure total blood flow (including primary and collateral vessels) to a distal extremity, as opposed to ultrasonography which is a better measure of single vessel anatomy and function. Additionally, strain gauge plethysmography is relatively inexpensive, light and easy to use, and requires minimal training for proficiency [61].

A.3.1.3. Indicator Methods

Several different types of indicators have been used in this technique. They can generally be divided into intravascular indicators (e.g. indo-cyanine green dye, Evans-blue, labeled microspheres, and labeled albumin), freely-diffusible indicators that pass through cell membranes (e.g. ^{133}Xe , heat, N_2O and H_2 gas), as well as small hydrophilic extracellular indicators (e.g. sucrose, insulin) which distribute in the interstitial space via small pores in capillary walls [55]. For an indicator to be indicative of blood flow it must not be retained or metabolized, and must fully mix with the blood. For thermodilution adequate mixing and heat transfer must occur, and for dye dilution the dye must bind to a carrier prior to distribution in the capillary network. Additionally, all these methods must be performed at steady state to allow accurate measures and injection of the perfusate must not interfere with this [67].

A distinction in indicator techniques is made between dilution (e.g. thermodilution and dye dilution) and perfusion techniques (e.g. ^{133}Xe clearance). Washout techniques are addressed under local blood flow techniques. Here I will outline dye- and thermo-dilution.

Dye dilution is based upon the principle that the concentration of an indicator is dependent upon the volume of blood flow through the artery [67]. This technique was originally developed for measurements of cardiac outputs but has been adapted for use in peripheral limbs. It is performed by infusion of a known concentration of dye at a specific rate followed by determination of the dye concentration (using a photo-densitometer) in blood sample withdrawn downstream. To remain valid it is assumed there is no recirculation of the mixture or samples are also drawn from a bi-lateral limb (working at the same level of activity) and the sample values adjusted accordingly. This technique is limited to steady state exercise but can be used during incremental exercise up to maximal intensities. The major disadvantages with this technique include, the invasive nature, the involvement of blood handling, and the possible need to replace fluids lost due to repeated sampling.

Thermodilution techniques are based upon a thermodynamic heat balance relationship, and knowing the specific heats of the blood, the infusate as well as the masses of the blood and the infusate [68]. Blood flow is determined from the temperature change in the investigation vessel during continuous infusion of cold saline. The temperature deflection is proportional to the temperature, rate of infusion, and rate of blood flow [55]. Measurements are taken from a thermostat inserted proximally in the artery from a catheter. This technique was also originally developed to measure cardiac output and adapted for peripheral measures. Bolus and continuous infusion techniques can be used at rest but the changes in intra muscular pressures during exercise (steady state) make only continuous infusions useful. The advantage of this technique is

that recirculation effects can be ignored and measures can be made more frequently than with dye dilution techniques. Although there are no alterations in blood volume, care must be taken to avoid overcooling of the measurement tissues. This technique is relatively inexpensive to purchase or to operate.

A.3.1.4. Doppler Ultrasound

For clinicians, the Doppler ultrasound probe has revolutionized investigations of peripheral vascular disease and blood flow. The underlying principle of the technique is that the frequency of reflected ultrasound waves is dependent upon the speed of flow of the blood from which the waves have been reflected [69]. Until 1970, Doppler ultrasound only provided information about blood flow rates. The introduction of pulsed Doppler allowed “ultrasonic arteriography” to be utilized [70]. This technique added depth to the information obtained in the returning signal. A short-burst of ultrasound is emitted and the crystal used to detect the returning ‘echo’ is only activated for a brief moment – a ‘time gate’. Depending upon the length of time between signal emission and receiver activation, the depth of the structure from which the returning signals enter the gate can be estimated. As this length is varied, a three dimensional map of the vessel can be constructed [69].

Duplex scanning is a more recent addition to Doppler’s capabilities. It combines real time ultrasonic imaging with Doppler flow measurements which have undergone sound spectrum analysis for visual display [71]. Even more beneficial is the development of color duplex scanning in which flow is represented on a real time image by a color scale, which can show velocity and direction. Additionally, as this technique became more widely utilized, waveform pattern recognition and subsequent analysis have also become commonplace.

In recent years external ultrasound imaging devices with high-frequency linear array transducers have been used to examine conduit arteries of the peripheral vasculature and their responses to various physical and physiological stimuli. Many of these studies utilize Doppler ultrasonography to obtain an image of the brachial artery at rest and following a period of forearm occlusion. The percent change in vessel diameter between the two is used as a marker of vascular function and may be a potential means of identifying patients with early atherosclerosis [6, 72]. Clearly, the appeal for using such a non-invasive, relatively painless, technique is enormous. Consequently, hundreds of papers have been published over the last 5 years using Doppler ultrasonography in this manner.

Despite the large number of cross-sectional studies the stability of this technique is not clear, and more importantly, convincing studies concerning the reproducibility of the technique are scarce. Several factors have been shown to influence arterial vasoactivity including time of day of testing [73], relationship to meals [74] and several hematological factors (see section 6). Satisfactory reproducibility has been shown in some studies despite no attempts to control for these factors and on limited variance components [6, 75, 76], along with unsatisfactory reproducibility found by others [77].

In an attempt to address these deficiencies our lab undertook a reproducibility study on 26 (41 ± 12 yr: range 22-57) individuals without overt signs of disease, acute medical conditions or on pharmacotherapy with known vascular effects. The experiments on these individuals were done under highly controlled conditions. Prior to the experiment subjects were instructed to fast and refrain from exercise for 12hr and alcohol for 48hr. Additionally, all ultrasound images were obtained at the same time of day, following 15min of supine rest in a quiet environment. Blood pressures were measured in the contralateral arm during the technique and blood draws

were performed immediately following vessel imaging in all participants at all visits. Subsequently, blood samples were examined for lipids, total protein, fibrinogen, glucose, electrolytes, viscosity factors, and homocysteine levels to ensure all subjects were within normal ranges and that no significant changes occurred between visits. No significant changes occurred between visits for blood pressures or for any of the hematologic variables. The study design was an incomplete model whereby intraclass correlation coefficients and covariance parameter estimates could be made for between and within days, ultrasound technicians, image readers and subjects. Average mean percent artery dilation difference for days was $1.91 \pm 1.07\%$, with an intraclass correlation coefficient (ICCC) of 0.9188 (95%CI = 0.7255-0.9823; p=0.0001). Average mean percent difference artery dilation for testers was $1.40 \pm 0.97\%$, with an ICC of 0.9394 (95%CI = 0.7562-0.9850; p=0.0001). Mean absolute difference in artery dilation for readers was $0.21 \pm 0.014\text{mm}$, with an ICC of 0.8982 (95%CI = 0.7650-0.9615; p=0.000). When components were combined days, testers, readers and subjects accounted for 14%, 19%, 0% and 43% of the total amount of variation, respectively. Although inter-reader image analysis proved to be the largest source of controllable variation, these data indicate adequate accuracy and reproducibility of this ultrasound technique under strictly controlled conditions [78]

In summary, it appears ultrasound duplex scanning and color-flow mapping represent the most accurate and versatile of all noninvasive vascular diagnostic techniques. The major limitations to these devices are their cost, complexity, and the relative lack of portability [79].

A.3.1.5. Magnetic Resonance Imaging

Recently, developments in magnetic resonance imaging phase contrast velocity mapping techniques have lead to the ability to calculate vessel diameters, blood flow volumes, and shear stresses in many different arteries [80]. This technique is based on the principle that hydrogen

nuclei in blood moving through a magnetic-field gradient accumulate a phase shift proportional to their velocity [81].

The advantages of this technique are that it is non-invasive, does not require the injection of any tracer, and may be especially useful in determining local blood flow in deep single vessels [82]. The technique has good image temporal and spatial resolution, but unfortunately it is very expensive to purchase and operate. An additional disadvantage is that the equipment is very cumbersome and therefore limits the type and intensity of exercise, which can be studied [55].

A.3.2. Local Blood Flow Techniques

A.3.2.1. Isotope Clearance

This technique is based upon the simple tenant that, clearance of a molecule or ion is purely dependent upon flow [83]. A tracer is injected intramuscularly and the washout clearance rate can then be observed as a measure of muscle perfusion [67]. Muscle blood flow can be calculated from the slope of the washout curve. The typical tracer for this technique is ^{133}Xe , which due to its lipophilic nature cannot become stored in fat tissue. Unfortunately, a problem with this technique is the non-linear relationship between the wash-out curves and actual blood flow. These errors could be contributed to different solubility constants of muscle, connective tissue and fat, isotope exchange between arterioles and veins and/or injection trauma [84]. Consequently, isotope clearance techniques may result in the underestimation of blood flow by up to 50%. A further problem is that due to the isotope injection trauma, it is recommended an interval of 10 minutes be undertaken prior to data acquisition. A further confounder is that, different blood flow values may be obtained by using different mathematical equations to convert the flow curves to empirical values [84]. In conclusion, this technique appears to have

value for measures of relative or serial changes in blood flow but considering the high costs of purchase and operation other options appear preferable.

A.3.2.2. Near-Infrared Spectroscopy

In 1977, Francis Jobsis demonstrated the feasibility of near-infrared spectroscopy (NIRS) to access the adequacy of oxygen delivery and utilization in living tissues [85]. More recently, using indocyanine green as a tracer, Colacino et al. were able to quantify blood flow through an area of duck brain [86]. Since this time the technique has developed to examine skeletal muscle blood flow at rest and during exercise. Comparisons of human forearm blood flow using NIRS and venous occlusion plethysmography show good correlation [87]. Recently, Boushel et al. used NIRS to measure muscle blood flow in the calf muscle during dynamic plantar flexion exercise and in the quadriceps muscles during knee extension and cycling [88-90].

NIRS is based upon the principle that near-infrared light (700-1000nm) passes through tissues with relative ease. The amount of light recovered after illuminating the tissue depends upon the degree of scattering by the tissues and the amount of absorption by chromospheres in the tissue. Currently, only hemoglobin, myoglobin and cytochrome-c oxidase are known to effect near-infrared light absorption. Using tracers, which absorb near-infrared light, it is possible, using the Fick principle, to produce flow estimates. The rate of accumulation of a tracer in a tissue is the rate of inflow minus the rate of outflow. Indocyanine green is an ideal tracer because it is non-toxic, soluble, has a relatively short half-life, and binds 95% to plasma proteins [91]. Indocyanine green is introduced rapidly into the venous circulation, where it is assumed to be evenly-mixed during passage through the heart and lungs, before entry into the arterial and tissue microcirculation. Blood can then be drawn from an artery to record an ICG curve. When the amount of tracer and blood flow in the injection artery are known, the blood flow in the

tissue region of interest can be estimated from the peak of the recorded dye curves. When the curve reaches maximum height, the measured tracer concentration will reflect blood flow in that region [91].

NIRS is sensitive for detecting progressive changes in tissue perfusion introduced by incremental exercise and for assessing perfusion differences between tissue regions [90]. In fact, an advantage of this system is the ability to track and differentiate blood flows and oxygen saturation's in different areas of microcirculation in the same muscle/limb [92]. Additionally, the ease of application, real time measures, and good signal integrity during movement also make this technique useful. Drawbacks to NIRS include the high cost of the equipment and the current limitation of a trade-off between light penetration to deeper tissues and spatial resolution. Improvements in the accuracy of this technique can be expected as NIRS technology continues to improve.

A.3.2.3. Microspheres

In 1969, the Rudolph and Heymann developed the microsphere technique to detect the distribution of blood flow within organs/tissues [93]. Since that time it has become the “gold standard” for measures of microcirculatory perfusion [94]. Since its inception, microspheres have been used in almost every field of cardiovascular research, and in a wide range of species and preparations varying from isolated organs to chronically instrumented animals on treadmills [95]. Additionally, microspheres is the only viable technique for measuring the distribution of blood flow among and within muscle of conscious animals during exercise [96].

The idea behind this technique is relatively simple. Radiolabeled spheres of 10-25 μ m diameter are infused into the left heart or aorta so they mix with the cardiac output and are distributed throughout the body in proportion to the flow rates of the individual tissues [97]. The

microspheres are just the right size to travel all the way to the arteriolar level and then get lodged in the small arterioles or capillaries. Under most conditions very few spheres return to the heart in the large veins, so most are trapped in the tissues on the first pass through. At the same time as infusion, a second catheter placed in the arterial tree distal to the infusion catheter is used to withdraw arterial blood at a known rate. This withdrawal is then used as a ‘ghost organ’ for calculating flows to various tissues by comparing its radioactivity to that of dissected organs after the experiment. Major technical assumptions which must be met are that the spheres are mixed freely and evenly distributed in the circulation and also that they become lodged in the tissues at the first pass. Additionally, care must be taken to ensure the microsphere mix and the drawing of blood samples does not interfere with the normal circulation [96]. The major disadvantage of this technique appears to be that the animal has to be harvested at the end of the trial to allow tissue dissection. Other disadvantages of this technique are the considerable expertise and facilities needed to administer the microspheres, the limited number of samples which can be drawn, and the possible changes in blood flow distribution caused by the technique itself [98]. Recently, non-radioactive techniques such as colored or fluorescent microspheres have been introduced, thereby reducing technician radiation exposure during repeated testing [95]. Despite these limitations the microsphere technique provides a powerful experimental tool for studying the distribution of blood flow within and among the muscles and other tissues during exercise [96].

A.3.2.4. Positron Emission Tomography

Due to the large number of tracers available for use with positron emission tomography, this technique can be utilized for non-invasive quantification of muscle perfusion, glucose utilization, fatty acid uptake and oxidation. It therefore represents an important tool to bridge the

gap between blood flow and metabolism [99]. Positron emission tomography measurements are based on the imaging of biochemical processes in vivo following the infusion of “natural” substrates labeled with short-lived positron emitting isotopes, and detection of γ -rays resulting from interactions between positrons and electrons [100]. Tracers such as ^{82}Rb and $^{13}\text{NH}_3$ have been used for blood flow measurements although H_2^{13}O is most widely used [100]. H_2^{13}O is an ideal tracer because it is freely diffusible and inert with respect to changes in tissue metabolism, and has a half-life of approximately 2 minutes. Using positron emission tomography blood flow mapping can be done using 2 methodologies, autographic or steady state. The autographic method uses radioactivity decay curves to mathematically determine blood flows and has been validated against plethysmography [101]. The steady state method involves the continuous administration of H_2^{13}O to achieve a radioactivity equilibrium, whereby tracer administration is in balance with tracer washout. The major benefits of this technique are the ability to link blood flow and metabolism, along with a greater ability to image possible heterogeneity of these distributions. The major disadvantages of positron emission tomography are the costs of purchase and operation of the equipment. Additionally, restrictions in imaging and data retrieval limit the type and intensity of exercise for which it may be utilized [55].

A.4. Circulatory Adjustments to Dynamic Exercise

At rest, blood flow to the human forearm and calf muscles is approximately 3-5 ml/100g tissue/min but may be 10 to 15 times higher immediately following intense muscle activity [102], demonstrating a huge increase in vascular conductance within these tissues. Additionally, considering limb skeletal muscle constitutes approximately 40% of the total body mass, central cardiovascular control mechanisms must ensure 1.2 liters/min of blood at rest,

increase to 20-25 liters/min at maximal dynamic exercise [102]. Once again the Fick equation forms a reference point for understanding the adaptations accompanying dynamic exercise.

$$\text{VO}_{2\text{peak}} = \text{HR}_{\text{peak}} * \text{SV}_{\text{peak}} * \text{a-v O}_{2\text{diffmax}}$$

Although it would be negligent to omit central cardiovascular changes in any review of cardiovascular adjustments to an acute bout of dynamic exercise, many authors have already comprehensively covered this topic. Therefore, the major focus of this chapter will address the adjustments occurring in the peripheral vasculature.

A.4.1. Central Adaptations

Central adjustments traditionally refer to heart rate, stroke volume, and the ability to provide driving pressure for tissue blood flow. This section will therefore mainly focus on the distribution of blood flow during dynamic exercise.

(i) Cardiac Output

Normal values for cardiac output at rest are approximately 5 l/min. This increases to approximately 25 l/min in healthy adults [103]. Cardiac output is the product of stroke volume and heart rate.

(ii) Stroke Volume

Stroke volume is the largest determinant between individuals $\text{VO}_{2\text{peaks}}$ and cardiac outputs. On average it ranges from approximately 70ml during upright rest to as high as 140ml during exercise. In elite endurance athletes however, exercise stroke volumes can be as high as 200ml. These increases occur despite the reduction in diastolic filling time from 0.55 sec at rest (70 beats/min) to 0.12 sec during maximal exercise (195 beats/min) [3].

In any review of the cardiovascular system it is essential to include the major factors which influence stroke volume. These include intrinsic factors such as cardiac contractility and extrinsic factors such as preload and afterload.

Increases in cardiac contractility can be attributed to an increase in heart rate, by a reduction in the diastolic relaxation phase and thereby reducing the time available for calcium clearance from the cell. Additionally, increased activity of cardiac sympathetic nerves and increases in plasma NE and Epi may also increase cardiac inotropy.

Ventricular filling pressure or preload is the degree of stretch on the muscle fibers of the ventricular wall prior to systole. According to the Frank-Starling mechanism this is positively correlated with stroke volume, because as a muscle fiber is stretched it causes an increase in tension and the ability to exert force.

Aortic pressure is the pressure to be overcome by the left ventricle in order for blood to be pumped out of the heart. This is known as afterload and determined largely by the compliance of the peripheral vasculature.

(iii) Heart Rate

In dynamic exercise heart rate increases with exercise intensity and oxygen consumption [104]. Typically, heart rates range from 70 beats/min at rest to approximately 200 beats/min at $\text{VO}_{2\text{peak}}$. These increases are controlled by a combination of parasympathetic nervous withdrawal and sympathetic nervous stimulation. (Figure A.6).

Most of the initial heart rate response (up to approximately 100 beats/min) is attributable to a reduction in vagal tone. Above 100 beats/min sympathetic activity becomes increasingly important [3]. This point of increased sympathetic activity is also important in alterations of basal vascular tone and the redistribution of blood flow to working tissues as described below.

Figure A.6. Circulatory Adjustments to Dynamic Exercise [3].

A.4.2. Peripheral Adaptations

(i) Arteriovenous Oxygen Difference

In dynamic exercise whole body arteriovenous oxygen difference ($a-v O_{2\text{diff}}$) increases with exercise intensity and oxygen consumption. Typically, $a-v O_{2\text{diff}}$ ranges from 5 $\text{mlO}_2/100\text{mlblood}$ at rest to approximately 15 $\text{mlO}_2/100\text{mlblood}$ at $\text{VO}_{2\text{peak}}$. These values are dependent upon many factors such as the concentration of hemoglobin in the blood, the oxygen binding capacity, alveolar PO_2 , pulmonary diffusion capacity, and alveolar ventilation [3]. During vigorous exercise hemoconcentration occurs due to increases in arterial pressures and a plasma volume shift in to the interstitium.

Arterial oxygen content of approximately $20\text{mlO}_2/100\text{mlblood}$ does not change with increasing exercise intensity but venous content falls considerably until approximately 85% of the oxygen is extracted at $\text{VO}_{2\text{peak}}$. Contributors to increased oxygen extraction include the redistribution of blood flow away from the splanchnic and visceral tissues in favor of working muscle (see below) which allows maintenance of a high oxygen extraction gradient between the blood and the non-working tissues. In fact, splanchnic tissues have been found to have extraction rates greater than 90% during prolonged exercise [105].

The working tissues also become more effective at extracting oxygen during exercise. At rest a small percentage of the total capillaries in a given tissue are open at any time. Consequently, the muscle fiber to capillary ratio is low, as is the mean transit time of the red blood cells through each open capillary, and oxygen diffusion distances to feed the muscle fibers are relatively high. These conditions are advantageous at rest because a high diffusion gradient for oxygen from the blood to the tissue is maintained.

During dynamic exercise the increase in muscle metabolism and oxygen demand causes a fall in tissue PO_2 , and a greater diffusion gradient from blood to the tissues. Although this contributes to increased tissue oxygen extraction the major determinants are an enormous increase in the number of open capillaries, providing a greater muscle fiber to capillary ratio and lower diffusion distances, along with higher red blood cell transit times.

(ii) General Redistribution of Blood Flow

To sustain physical activity for more than a few seconds requires dramatic circulatory adjustments to ensure adequate perfusion of the exercising skeletal muscle as well as of vital organs such as the heart and brain [106]. In fact, the demands of exercise in a large volume of muscle mass can approximate maximal cardiac output [107]. This increased demand presents a significant challenge to blood pressure regulation. The cardiovascular system must co-ordinate these opposing demands to produce an optimal pattern of vasodilation and constriction designed to maximize performance while defending blood pressure.

As yet, the exact mechanisms involved in this integrated process are not entirely known. It is established that hemodynamic adaptations are mediated by a decrease in parasympathetic and an increase in sympathetic outflow controlled both by feed-forward from increased central drive and by feed-back arising from mechanically and metabolically sensitive afferent nerve endings in the contracting skeletal muscles.

A relatively easy and widely utilized method to approximate sympathetic nervous activity is plasma norepinephrine. The relationship between plasma NE and HR is relatively linear above 100 beats per minute. Additionally, the relationship between plasma NE and the decrease in splanchnic (Figure A.7) and renal blood flow is close and relatively predictable [109]. This demonstrates the reproducibility of the HR to splanchnic (and renal - dashed lines) blood flow

Figure A.7. The Relationship between Splanchnic Blood Flow as Percent of Resting Values and Heart Rate during Exercise and Other Stresses [108].

with regression lines derived from approximately 200 observations during supine rest combined with lower body negative pressure or heat stress [108, 110], and approximately 500 observations during exercise, exercise plus heat stress, and exercise before and after physical conditioning [111]. Interestingly, the effects of parasympathetic withdrawal on splanchnic blood flow are poorly documented.

An examination of blood flow distributions to other tissues was performed using radioactive microspheres by Horimer et al. (1983), during mild dynamic leg exercise in 5 chair restrained baboons (Figure A.8) [112]. Blood flow to the exercising leg muscles increased by $585 (\pm 338)\%$ and decreased by $38 (\pm 4)\%$ to the torso, by $38 (\pm 6)\%$ to the limb skin, by $30 (\pm 10)\%$ to the non-exercising muscles, by $16 (\pm 2)$ to the kidneys, and by $21 (\pm 9)\%$ to the splanchnic region. Hohimers conclusion was during short dynamic exercise blood flow was redistributed away from skin, fat, non-working muscles and visceral organs to supply the needs of exercising muscles [112].

A.4.3. Peripheral Adaptations of Blood Flow at the Onset of Dynamic Exercise.

Much of what is known about skeletal muscle blood flow is taken from studies performed at rest or during steady state exercise [113]. Interestingly, the key time period to be examined maybe the transition form rest to steady state when central and peripheral control mechanisms often compete for vascular control. The prevailing view of skeletal muscle exercise hyperemia is that it is primarily mediated by local control mechanisms and that central control systems are superimposed on the dominant local control systems (Figure A.9) [114, 115].

Figure A.9 outlines the major established factors involved in peripheral skeletal muscle blood flow (q). These mechanisms include mechanical (top row) and non-mechanical (bottom row) influences that act directly on vascular smooth muscle to reduce (-) or increase (+) vascular

Figure A.8. Redistribution of Blood Flow during Mild Dynamic Exercise in Conscious Baboons [112]

Figure A.9. The Major Established Factors Involved in Peripheral Skeletal Muscle Blood Flow. Limb blood flow (q) is determined by the interplay between several mechanical (*top row*) or nonmechanical (*bottom row*) factors that act directly on vascular smooth muscle to reduce (-) or increase (+) limb vascular conductance (VC). Limb flow will also increase if the pressure gradient ($[\Delta P]$) across the capillary bed following contraction is enhanced by activation of the muscle pump (MP) or by an increase in perfusion pressure (PP). Further, VC can be altered by the sympathetic nervous system (SNS) following integration of opposing reflex signals arising from the baroreflex (BR) and chemoreflex (CR) and from higher brain centers; the role of central command in blood flow control is not clearly defined. In turn, some factors may act indirectly by augmenting ([black small square]) or attenuating ([white circle]) the effect of other regulatory agents (*dotted lines*). The combination of these factors results in a net change in limb blood flow. CNS, central nervous system; PG , prostaglandins; NO , nitric oxide; $[\beta]AR$, beta-adrenergic receptor; $[\alpha]AR$, alpha-adrenergic receptor; P_i , inorganic phosphate; La^- , lactate; K^+ , potassium ion; ACh , acetylcholine [113].

conductance (VC). Limb blood flow is also dependent upon the pressure gradient (ΔP) across the capillary bed following contraction. This is enhanced by activation of the muscle pump (MP) or by an increase in perfusion pressure (PP). The sympathetic nervous system can also influence VC, following the integration of opposing reflex signals originating from the baroreflex (BR) and chemoreflex (CR) and from higher brain centers. Some factors may act indirectly by augmenting () or attenuating () the effect of other regulatory agents (*dotted lines*) [113]. Unfortunately, each of these factors may have a different proportional contributions to VC across the time course of the rest to work transition and, to complicate the issue further, at different points in the vascular tree [38, 39]. This section will attempt to summarize the current literature in these areas to provide a clearer picture of peripheral skeletal muscle blood flow during dynamic exercise.

It is apparent that the time course of rest to work peripheral blood flow transition is biphasic, with a tremendous increase during the initial 7 seconds (phase 1) followed by a slower rise occurring about 20 seconds until steady state is achieved (Figure A.10). The phase 1 rise is so rapid it argues against the influence of non-mechanical factors.

A.4.3.1. Mechanical Contributions

i) The Muscle Pump

Mechanical interactions between contracting and relaxing skeletal muscle and blood vessels may augment blood flow to these tissues in several ways [116]. Blood flow is determined by the interaction of the total energy gradient driving blood and the impedance of the vascular network (Figure A.9). The pressure gradient (ΔP) is the product of perfusion pressure (PP) (arterial minus venous pressure) and the resistance to flow. In most tissues mean arterial and venous pressures are normally maintained within a narrow range. Thus, blood flow control

Figure A.10. The Rapid Biphasic Increase in Limb Blood Flow at the Onset of Exercise
Limb blood flow increases rapidly at the onset of exercise in a biphasic manner. An initial plateau following 5-7 s of exercise (Phase 1) gives way to a more slowly increasing segment following ~20 s (Phase 2) toward a new steady-state level [113].

is accomplished by variations in vascular resistance which result from changes in the caliber of resistance vessels [117]. Skeletal muscle contraction causes dramatic effects upon the caliber of the vessels within muscle tissue and the term “muscle pump” refers to contraction-induced, rhythmic propulsion of blood from skeletal muscle vasculature, which facilitates venous return to the heart and perfusion of skeletal muscle [115]. The muscle pump is proposed to act via two mechanisms [117, 118]. Firstly, the contraction phase of skeletal muscle causes compression of the veins, which imparts kinetic energy to the blood, causing it to move out of the compressed segments. Due to the valve orientation in the venous system, blood can only flow towards the heart. The refilling of this pump mechanism occurs at muscle relaxation as blood refills the veins [117-119]. Secondly, the muscle pump alters the pressure gradient (ΔP) across capillaries by lowering venous pressure in the venules and deep small veins during the relaxation phase of the contraction cycle [117, 118]. The veins are attached to the muscle tissue that surrounds them and are therefore pulled open when this muscle relaxes. Pressure is lowered by this increase in venous volume and by the fact that it should already be empty from the previous contraction [118]. Interestingly Folkow et al. demonstrated that arterial inflow occurred throughout the relaxation period, yet there was no femoral venous outflow during relaxation, suggesting venous segments may not refill instantaneously [119, 120]. This could be interpreted as sequential filling of venous compartments following contraction, from the first venous valves in the small veins to the systemic veins. If the next contraction is sufficiently delayed the venous bed will completely refill. If not, venous pressures will remain decreased for the next relaxation phase [117]. The two actions of the muscle pump are only effective during rhythmic contractions, whereas tetani results in an increase in resistance to flow [115, 117, 118].

Early research supporting the muscle pump hypothesis demonstrated blood flow in a maximally dilated forearm at rest could be doubled during rhythmical contractions [121, 122]. This work was confirmed by Laughlin et al, (1987), during treadmill exercise in rats where they estimated up to 30% of the total perfusion of the soleus and up to 60% of the red portion of the gastrocnemius muscles respectively was due to the muscle pump [118].

In an effort to distinguish the contributions of the various stages of the muscle pump Folkow et al. examined the calf muscle blood flow during heavy plantar flexion exercise in individuals in supine or upright postures. This logic was a larger venous pressure prior to a contraction would lead to a larger change in the pressure gradient and flow following contraction. Blood flow was 60% higher in the upright position illustrating the contribution of a positive hydrostatic pressure (or venous suction) on the efficiency of the muscle pump [119].

Sheriff et al. ran instrumented, conscious dogs on a treadmill and found if they maintained a constant cardiac output or mean arterial pressure, the rate of muscle contraction was more important than workload per-se in the early blood flow adaptation phase. Workload became more influential after approximately 10 sec of work, indicating a possible metabolite influence [123].

Leyk et al. challenged this theory in human subjects using Doppler ultrasound to measure femoral blood flow during calf contractions. Their work showed a greater hyperemic response when the participant was seated upright than when they were positioned with the legs above the head [124]. This supports the muscle pump hypothesis because if the limb is above the heart venous volume is reduced as blood flows back to the heart due to gravity. Leyk et al. also claimed the magnitude of venous and hemodynamic pressure changes between positions could not completely account for the changes in limb blood flow. Unfortunately, they didn't account

for changes in central hemodynamics brought about by positional manipulations [113]. In 1998, Shoemaker et al, examined forearm flows 5 sec after initiating rhythmic contractions [125]. They observed a 40% increase in hydrostatic pressure when the arm was positioned below compared to above the heart, and a 50-100% increase in flow. Additionally, they reported a matching of the time course of blood flow to the absolute work rate when workload and contraction rate were manipulated. This was interpreted as evidence of a coupling of flow to metabolic needs and/or fiber recruitment patterns.

As described above the contribution of the muscle pump in the early phase of blood flow increases in exercise is well documented. It remains the major contributor to ensure tissue beds receive adequate blood to meet the needs of increased workload. Unfortunately, two major issues remain unclear. Firstly, what is the contribution of vasodilator influences to these early changes? Secondly, how does this overall system vary between different muscle types?

Shoemaker et al. [125] was not the first investigator to hint at a possible metabolite effect to cause phase 1 vasodilation. In 1996 Tschakovsky et al.[126] used a mechanical forearm pneumatic cuff which could be rapidly inflated for 1 sec and deflated versus a single dynamic 1 sec forearm contraction. In addition, the experiments were performed with the arm above or below the level of the heart. Blood flow was measured on a beat by beat basis using Doppler ultrasound. During the cuff inflation/deflation, blood flow only increased when the arm was positioned below the heart, whereas during voluntary contraction blood flow increased in both conditions. Additionally, the arm below the heart blood flow response was greater with the contraction condition than with the cuff [126]. The authors concluded that this suggests the influence of other non-mechanical factors. Another possible explanation could lie in differences between the pumping efficiencies of the cuff compared to muscle.

ii) Myogenesis

Any influence other than the muscle pump mechanism must have the ability to influence vascular smooth muscle fast enough during the contraction phase of this dynamic exercise to allow greater dilation during the relaxation phase. This theory is appealing, as it would allow for a more efficient matching of flow to the working muscle fibers even in the same muscle bed. Due to this rapidity of the effect the mechanical nature of myogenesis would appear to be a possible candidate. Unfortunately, this theory has received little experimental support. In 1981, Bachus et al. found that rhythmically changing the extravascular pressure on in canine gracilis muscle, in vitro, failed to produce changes in vascular response [127]. This work was confirmed by Sheriff et al. by manipulating arterial pressure in exercising dogs. The initial increase in hindlimb vascular conductance after the initiation of running was unaffected by both increases and decreases in transmural pressure [123]. These findings in conjunction with Tschakovsky's work [126] appear to negate a myogenic contribution to Phase 1 increases in blood flow.

A.4.3.2. Other Contributors

i) Neurogenic Influences

An attractive alternative to myogenic influences, given the time constraints, is some kind of neurally controlled vasodilation. After all, β -adrenergic and muscarinic receptors are present on the skeletal muscle arterial vasculature [128], and blood flow to muscle can increase, as part of an anticipatory response, prior to exercise activity [129] (which must be prior to metabolic or mechanical influences). The sympathetic nervous system could initiate skeletal muscle hyperemia through an increase in sympathetic cholinergic nerve activity or a withdrawal of sympathetic adrenergic stimulation [115].

The existence of sympathetic cholinergic-mediated vasodilation in the skeletal muscle vasculature is an attractive hypothesis because not only would direct stimulation cause dilation, a possible acetylcholine spillover effect from the neuromuscular junctions of active tissue would provide a link between muscular contraction and blood flow [130]. Studies using β -blockade have shown some conflicting results. Unfortunately, those demonstrating a reduction in blood flow tended to use non-selective β -blockade, causing a systemic reduction of heart rate and blood pressure, thus making it impossible to attribute limb blood flow reductions to local mechanisms [128, 131-133]. Studies finding no effect of β -adrenergic blockade are more numerous and appear to be more valid. As early as 1969, Bolme and Novotny administered atropine (a cholinergic muscarinic receptor blocker) to conditioned treadmill running dogs. The atropine administration obliterated the anticipatory response (to a verbal cue), but did not effect the magnitude of muscle hyperemia at the onset of exercise [134]. In 1998, Buckwalter et al. followed up their earlier studies by trying to differentiate whether β -adrenergic or muscarinic receptors were involved in skeletal muscle vasodilation at the onset of exercise [130]. Using instrumented mongrel dogs running on a treadmill they infused via the femoral artery either; (a) 1mg of propranolol (a non-selective β -antagonist); (b) 500 μ g atropine (a muscarinic antagonist); (c) both 1mg of propranolol and 500 μ g atropine or; (d) a control saline solution. Immediately prior to exercise the appropriate solution was infused into one leg. The dogs then ran on a motorized treadmill at 3mph/0% grade. Blood pressure and blood flow were monitored in both the infusion and control legs. The advantage this study had over its predecessors was the use of a local infusion of small doses of solutions creating a functionally isolated limb, coupled with the continuous recordings of blood flow using locally located flow probes. No changes in the time course or magnitude of blood flow responses were observed, indicating neither β -adrenergic nor

muscarinic receptors are required for immediate skeletal muscle vasodilation at the onset of exercise [130].

The rejection of a neural mechanism is also supported by lumbar sympathectomy studies in rats [135], where initial hyperemic responses were similar to control animals. Interestingly, steady state blood flows were actually higher in the sympathectomized animals.

Finally, in 1990, Joyner et al. used posture to alter sympathetic nerve activity in humans. They found no difference in forearm hyperemia at the onset of exercise in the upright posture (when sympathetic nerve activity is high) compared to the supine position where nerve activity is low [136].

ii) Metabolites

Having considered mechanical (muscle pump and myogenesis) and neurogenic contributions to phase 1 blood flow increases we must now consider substances released from the tissues or blood which could initiate vasodilation. A variety of substances with vasodilator properties are released from active skeletal muscle. These include ions, metabolites, and gases such as carbon dioxide, or oxygen, and more recently nitric oxide [116]. The mechanisms for metabolic dilation must involve either a direct effect on vascular smooth muscle tone or by interference with adrenergic constrictor influences [113]. The obvious attraction to this theory is the again the matching of blood flow to the working, metabolite producing fibers/tissue. Indeed, in longer duration work metabolites are undoubtedly major contributors in this matching process.

iii) Potassium

The most likely culprit for metabolite induced phase 1 increases in blood flow is potassium ions (K^+), which are known to cause vasodilation at relatively low concentrations [137]. K^+ is released into the interstitial fluid as a result of skeletal muscle repolarization

following contraction and its accumulation can mirror hyperemia to that muscle. In twitch contractions a dilatory threshold can be reached in approximately 10-20 seconds and peak levels occur at about the 2-minute point [115]. Unfortunately, the effect on the smooth muscle membrane $\text{Na}^+\text{-K}^+$ ATPase pump, which mediates the K^+ effect, takes considerably longer. Smooth muscle relaxation occurs via a reduction of the Ca^{2+} content of the cell. This can occur by a reduction in Ca^{2+} influx into the cell or by an increase in Ca^{2+} efflux via the $\text{Na}^+\text{-Ca}^{2+}$ exchange pump. This process can take up to 15 seconds from time Na^+ reaches dilatory threshold concentrations [138, 139].

Experimental evidence questioning the role of K^+ in early vasodilation comes from Chen et al. (1972). They measured canine hind limb perfusion pressures as an index of vascular tone, during contractions prior to and following treatment with ouabain. Ouabain treatment had no effect on the magnitude of fall in resistance with contractions [140]. In addition, Hnik et al, (1976) found twitch and tetanic stimulation of the cat gastrocnemius caused substantial hyperemia with only small elevations in interstitial K^+ levels during the initial 10 to 20 sec of exercise [141]. Wilson et al. (1994) confirmed these findings in the contracting human forearm, whereby substantial hyperemia occurred with only small increase in venous K^+ concentration [142].

iv) Hydrogen Ions

Hydrogen Ions (H^+) production is another potential mediator of early hyperemia. Unfortunately, animal and human experiments [143] have demonstrated an initial increase in muscle pH at the onset of contractions. Although this is followed by an eventual decrease in pH, the time course is too slow to implicate H^+ in phase 1 blood flow changes [143, 144].

v) Adenosine

Adenosine is a byproduct of the hydrolysis of ATP and as a result accumulates in the interstitium. Skeletal muscle concentrations of adenosine during early exercise are usually relatively low (when compared to cardiac tissue). This is probably related to the presence of adenosine deaminase, which is much higher in skeletal muscle tissue [145]. However, while adenosine is predominantly formed in type I fibers during sub-maximal exercise, dilation also occurs in type II fibers [146].

vi) Other Metabolites

Oxygen and carbon dioxide can also be eliminated as possible candidates for early vasodilators. Arterial O₂ content of the blood appears, under most circumstances, to be in excess of metabolic needs[147] and experiments that manipulate the partial pressure of O₂ appear to have little impact on hyperemia [148]. Additionally, CO₂ produced by contracting muscle is unlikely to accumulate to a sufficient partial pressure to account for their dilation [102].

In conclusion, it is unlikely that the early dilatory response is related to the metabolism of the recruited fibers [113]. The blood flow hyperemia response can be dislocated from the metabolic demand by alterations in workload [146], and by paralyzing the muscle with curare [149, 150]. In addition, exercise training may also disassociate this relationship. Short-term endurance training results in faster conduit artery blood flow response[151] and tighter metabolic coupling[152, 153] when it should also reduce the production of metabolites at the onset of contractions.

A.4.4. Increases in Hyperemic Blood Flow from Phase II to Steady State

The current consensus suggests the phase II increase in blood flow at the onset of exercise begins approximately 15-20 sec after onset and is predominately feedback regulated,

unlike phase I [113]. The principal regulatory mechanisms involved are central command and peripheral feedback [154]. They defend systemic blood pressure and provide adequate blood flow to working tissue via increases in cardiac output and venous return, increases in vascular resistance in inactive tissues, and by decreases in vascular resistance in the active skeletal muscles [84, 155]. The purpose of this section of the literature review is to examine the influence of these factors at the peripheral vasculature up to and including steady state exercise.

A.4.4.1. Sympathetic Control

Central cardiovascular control mechanisms are primarily for the maintenance of systemic perfusion whereas local vascular control mechanisms are primarily for the maintenance of tissue homeostasis. During rest sympathetic tone is relatively high and contributes to basal vascular resistance, yet in exercise sympathetic outflow increases dramatically and would increase resistance if not mediated at the local level. An example of this at low exercise intensity was shown by Peterson et al, (1988) in rat hindlimbs after undergoing acute lumbar sympathectomy [135]. Increases in blood flow were observed indicating mediation of sympathetic vasoconstriction by local control mechanisms possibly including, metabolites, endothelium derived factors, propagated signals and the muscle pump effect [156]. This phenomenon can also be observed in humans with autonomic failure as they cannot maintain arterial blood pressure during light supine exercise [157].

The concept that muscular activity may alter the responsiveness of the vasculature to vasoconstrictor substances was referred to as ‘functional sympatholysis’ by Remensnyder et al. (1962) [158].

“In essence, the term ‘functional sympatholysis’ describes the interaction between a vasoconstrictor stimulus (i.e. sympathetic activation) and a vasodilator stimulus (i.e. muscle contraction). The net effect of this interaction or the degree of sympatholysis, will vary depending on both the strength of the vasoconstrictor stimulus and the intensity of the muscle contraction” [106]

Differences in the degree of sympatholysis between arteries of different caliber have advanced the concept of heterogeneity of distribution of α_1 and α_2 receptors within the microcirculation. Large resistance arterioles are served by both α_1 and α_2 receptors whereas small nutrient arterioles are served by predominantly α_2 receptors [159]. This becomes more intriguing when we consider the α_2 receptors are much more sensitive to metabolic inhibition than the α_1 receptors. Therefore the response to sympathetic nerve stimulation [160, 161] and the susceptibility of sympathetic vasoconstriction to inhibition by muscle fiber activity (appears greatest in distal versus proximal arterioles.

Additionally, animal studies have demonstrated discrepancies between muscle fiber type responses to sympathetic stimulation. For example, Thomas et al. (1994), showed preserved sympathetic vasoconstrictor response to nerve stimulation during maximal contractions of the rat soleus, but not the gastrocnemius-plantaris. At rest and during low intensity work, stimulation reduced blood flow to both tissue beds. When the stimulation was doubled to the maximally contracting gastrocnemius-plantaris, partial attenuation of blood flow occurred [162]. These findings demonstrate that the net vascular response is dependent upon the tissue type, the strength of the sympathetic stimulus and the degree of metabolic stress placed upon the tissue by muscle contraction [106].

The evidence for exercise induced sympatholysis in humans is somewhat conflicting. These discrepancies can be related to the different assessment methodologies used to examine the microcirculation, but more realistically upon the complex nature of factors contributing to the

sympatholytic response. These include the mode, intensity and duration of the activity, the active muscle(s) fiber type composition and the nature and intensity of the sympathetic stimulus [106].

Nevertheless, near infrared spectroscopy has revealed a differentiation in tissue oxygenation between active and inactive tissues during high-intensity handgrip exercise. Additionally, at 20-33% MVC handgrip exercise manipulation of the sympathetic stimulus (using lower body negative pressure) had no effect upon the oxygenation status of the active tissues but reduced oxygenation in the inactive tissues. This preservation of oxygenation was abolished when handgrip work was less than 10-20% MVC. These results can be interpreted as different intensities are needed to activate sympathetic vasoconstriction (10-20% MVC) than those required to initiate metabolic sympatholysis (>33% MVC) [163]. Attenuated noradrenaline-induced vasoconstriction of the brachial artery has also been reported during handgrip exercise using magnetic resonance imaging [164].

A.4.4.2. Metabolic Control

The metabolic hypothesis proposes tissue metabolism and arteriolar smooth muscle constitute a local control system that provides the coupling between blood flow and tissue energy and nutritional requirements [114]. Metabolites from active skeletal muscle diffuse into the interstitium and resistance arteries, causing vasodilation and capillary recruitment. The increased blood flow and oxygen extraction that result from metabolic vasodilation increase oxygen supply to a level compatible with tissue oxygen demand [156]. Vasoactive substances proposed to be involved in this dilation include H^+ , K^+ , Pi^+ , CO_2 , adenosine, adenosine nucleotides, lactate, TCA intermediates, vasoactive peptides and prostaglandins [84, 165]. The difficulty in identifying the contribution of each of these metabolites individually to the

vasodilative and steady state flow is that different factors may have greater influence at different states of exercise and in different locations in the vascular tree. In fact, in 1975, Skinner pointed out that they work in an interactive, non-additive way so that the combined effects outweigh any single effect [166], which obviously confounds the reductionist nature of most conventional approaches.

Without the time constraints of phase 1 dilation non-mechanical mechanisms appear to have a much more feasible influence on vasodilation. A good amount is known about the long term angiogenic adaptations to muscle metabolism under conditions such as physical training, hypoxia and altitude exposure, yet after more than 100 years of research the chronic mediators of dilation are not known [167]. This section will review the major substances involved in this process.

i) Potassium Ions

Potassium ions may play a role in early phase II vasodilation due to skeletal muscle depolarization but appears to be less important as exercise continues because the arterio-venous difference for K^+ diminishes [116, 156]. Additionally, inter-arterial infusions of K^+ necessary to invoke forearm vasodilation also cause pain, which does not occur in mild exercise [168].

ii) Adenosine

Adenosine is a potent dilator when given exogenously to skeletal muscle and with a very short half-life would appear to be ideal as a key metabolite for blood flow regulation.

Unfortunately this makes it difficult to quantify in the interstitium in terms of its ability to reach and bind to purinergic receptors [116]. Experimental reports in animals are often conflicting. For example, Klabunde (1988) found infusions of adenosine deaminase into rats did not alter skeletal muscle blood flow or perfusion at rest or during low intensity treadmill running [169].

In contrast, Metting et al. (1986), found impairment in exercising canine hindlimb vasculature to adapt to decreases in perfusion when adenosine receptors were blocked [170]. During leg exercise in humans, sufficient to raise femoral blood flow to $5\text{-}6\text{ L}\cdot\text{min}^{-1}$, a further infusion of adenosine (and inflation of a cuff below the knee) enhanced blood flow by a further $2\text{-}3\text{ L}\cdot\text{min}^{-1}$ [171].

Furthermore, research utilizing dipyridamole (an inhibitor of adenosine uptake by tissues) showed differences in skeletal muscle fiber type dilation, with greater blood flows in slow twitch fibers during sub-maximal exercise [172]. Schwartz and McKenzie (1990), using adenosine deaminase in exercising felines, supported this theory of differential release of adenosine during sub-maximal exercise. They reported diminished hyperemia in the soleus but not in the gracilis [173]. In humans, it appears all fiber types may produce adenosine during exercise intensities above $\text{VO}_{2\text{peak}}$ or at constant or limited blood flow perfusion [169, 172, 174].

It is apparent adenosine fulfills the criteria to be a dilator in voluntary contraction. It is produced in the interstitium in functionally significant amounts in relation to exercise intensity. Thus it could be the link between muscle contraction, metabolic rate and vasodilation. This does not mean it has to be the only link between these processes. In a inventive experiment devised to investigate the contribution of adenosine to vasodilation Hester et al. (1982), infused adenosine into an isolated canine hindlimb model at super-physiologic doses for prolonged periods in an attempt to ‘saturate’ the adenosine-mediated dilator system. A marked rise in flow occurred, which despite continued administration of adenosine, returned slowly to baseline. Interestingly, there was still a rise in blood flow and dilation when contractions were superimposed upon the adenosine infusion [175]. Therefore, vasodilation during muscle contraction can also occur independent of adenosine mediated mechanisms.

A.4.4.3. Nitric Oxide and Prostaglandins (Endothelial control)

The discovery of the vascular endothelium and endothelial derived relaxing factors [25], has lead to a plethora of research revealing its importance in the regulation of vascular smooth muscle tissue and peripheral blood flow control [37, 39, 115, 176, 177]. In fact, the endothelium is implicated in constricting and dilating influences as well as, structural adaptations of the vasculature and is responsive to both physical (shear stress) and chemical stimuli (NE, acetylcholine, bradykinin, vasopressin) in the vascular tissue and in the blood [113, 156, 178]. Other possible stimulators of nitric oxide release include metabolites (which trigger specific receptors) [179], and sympathetic cholinergic nerves [180]. Also nitric oxide may be released directly by nitroxidergic nerves [181], hemoglobin (see figure A.11) and from tissues, such as skeletal muscle [182]. Vasoactive substances currently understood to be released by the endothelium include nitric oxide (NO) [183], prostacyclin (PGI₂) [184], and endothelial derived hyperpolarizing factor (EDHF) [185].

The most investigated of the endothelial relaxing factors is nitric oxide. It appears Ca²⁺ is an essential cofactor to the enzyme nitric oxide synthase, which converts the amino acid L-arginine to citrulline and nitric oxide. Nitric oxide diffuses through to the underlying smooth muscle cells and activates the enzyme guanylate cyclase to convert guanylate tri-phosphate to cyclic-guanylate mono-phosphate [186]. Cyclic-guanylate mono-phosphate performs many cellular functions by activating specific protein kinases that result in relaxation [187]. These actions include increased Ca²⁺ extrusion from the cell, increased Ca²⁺ uptake into the sarcoplasmic reticulum, and activation of ion channels which hyperpolarizes the smooth muscle cell [37, 187]. This results in vasorelaxation and dilation of the arterial vasculature. The enzymes that produce NO have been divided into several categories and nomenclatures the most

Figure A.11. Mechanisms of Nitric Oxide Release [179]

basic of which is constitutive-NOS (cNOS) because it is freely expressed and needs free cellular calcium. This form also responds to shear stress and antagonists in contrast to the inducible form (iNOS). iNOS is induced by cytokines and appears to be always tightly bound to calcium and calmodulin [32, 178]. Further distinctions have been made based upon chronological order and the tissue in which the enzyme was first isolated [188]. For the purpose of this review, although cNOS is probably the most applicable, we will not distinguish between isoforms because the literature is currently unclear in its definitions.

Our understanding of NO production mechanisms has led to several methods of investigation of the role of NO in arterial vascular control. For example, guanylate tri-phosphate can be activated directly by the vasodilators nitroglycerin and sodium nitroprusside. Additionally, several varieties of L-arginine analogues have been developed to competitively inhibit nitric oxide synthase the most popular of which is N^G-monomethyl-L-arginine (L-NMMA) [179]. L-arginine supplementation has also been employed.

Although the majority of research in this domain is focused on NO it is important to briefly review the role of prostacyclin and EDHF in vascular control during exercise. PGI₂, like NO can be stimulated by a variety of factors and is derived from the action of cyclooxygenase on arachidonic acid. Its release by the endothelial cell causes a relaxation in the smooth muscle via and increase in cyclic adenosine monophosphate (cAMP) which, in turn, activates specific protein kinases [189]. Koller et al, [184] demonstrated the importance of PGI₂ to arteriole vasodilation, following an increase in blood flow, in a series of isolated rat preparations [184]. In humans, pretreatment with 1200mg/dl of ibuprofen for 2 days reduced PGI₂ synthesis but had no effect on brachial flow response to 15 seconds of handgrip exercise. Therefore, PGI₂ may not be essential in establishing the magnitude or time course of blood flow in the rest to exercise

transition [190]. The nature of EDHF is not yet clearly understood. It appears to be released by the stimulation of endothelial M₁-muscarinic receptors and probably acts via the activation of a K⁺ channel [30].

Research techniques to investigate the role of NO in peripheral blood flow have employed several different types of preparations ranging from isolated arterioles to whole animal studies [186]. The literature consensus is that NO plays an important role in blood flow at rest. In isolated preparations King et al. reported a decrease of approximately 25% in hindlimb blood flow with the inhibition of NO in dogs [191]. Similar findings are reported in cats hindlimbs [192] and human forearm muscles [44].

The influence of NO during dynamic exercise is less clear. For example, Persson et al. found no effect of L-NMMA on isolated rabbit tenuissimus muscle during twitch contractions [193]. Similarly, Barclay and Woodly, reported no effect on blood flow in canine gastrocnemius muscle during twitch contraction, despite an effect at rest [194]. In humans, Wilson and Kapoor, found identical blood flow responses to exercise before and after NO blockade [195].

There appear to be three points of contention, which cloud the issue and prevent any clear interpretations for the role of NO. Firstly, the caliber of the arteriole may impact the effect of NO. For example, Hester et al. presented evidence that NO is important in the 1-minute hyperemic response and dilation of first and second but not third order arterioles during twitch stimulation of the hamster cremaster muscle [196].

Secondly, the intensity of the balance between the degree of the blockade and the exercise stimulus appears to be important in determining the role of NO. For example, Gilligan et al. found human forearm blood flow response to handgrip exercise, at 45% MVC, was significantly reduced following NO blockade by up to 26%. More interestingly, the degree of

reduction in flow was related to the degree of NO blockade [197]. Additionally, O’Leary et al. measured blood flow through the terminal aorta of the dog during treadmill running before and after L-NMMA. They found significantly reduced flows at rest and following moderate exercise but no difference at higher workloads [198].

Finally, the composition of the muscle in which the artery serves may have an influence on the blood flow response elicited. For example, Shen et al. accidentally discovered differences in blood flow reductions in three locomotorary muscles in exercising dogs with the infusion of L-NMMA [199]. Subsequently, Hirai et al. found a linear relation between attenuation of exercise-induced hyperemia and the fiber composition in rats during moderate intensity treadmill running. Muscles with the highest content of high oxidative (slow and fast twitch) fibers exhibited the greatest reductions in blood flow [200]. They concluded that NO contributes to skeletal muscle blood flow response to a given level of submaximal exercise and that the NO pathway contributes to the differential blood flow response found within and among the different locomotor muscles of the rat during exercise [200, 201].

Interestingly studies by Shen et al. in conscious exercising dogs, have shown that the vascular beds which normally experience decreased blood flow during exercise, such as the splanchnic and renal circulations, experience even greater vasoconstriction and vascular resistance following NO blockade [199]. These findings prompted them to hypothesize that the primary stimulus to overcome central command is metabolic, whereas NO promotes the fall in vascular resistance but is not responsible for the increase in blood flow. NO therefore acts as an amplifier [188, 199, 202].

“In this schema, metabolic dilation causes an increase in blood flow and shear stress and then the increased shear stress stimulates NO production by the endothelium and adds to the fall in vascular resistance.” [188]

This is consistent with the observation that vascular dilation appears to be initiated in the smallest microvessels [203] with the dilatory signal progressing upstream to include feed arteries. Therefore, the distribution of flow is regulated by the microvessels and the larger vessels adjust the magnitude of flow [51, 204]. In fact, Doppler observation of the brachial artery has shown progressive dilation for handgrip exercise beyond 60 seconds, independent of arm position [190]. Although, the distinction if dilation during exercise is caused by ascending vasodilation, by flow mediated mechanisms, or by both is currently unclear.

A.4.4.4. Other Factors

Experiments using hyperoxia or hypoxia suggest a closely coupled inverse relationship between arterial O_2 or PaO_2 content and blood flow at sub-maximal exercise intensities [148, 205]. Interestingly hemoglobin concentration has a similar relationship [206]. This suggests these factors may play a role in the regulation of muscle blood flow [207].

The possible effects of PaO_2 could be mediated via an increase in adenosine production and by the opening of ATP-sensitive K^+ channels (see earlier) [208]. Unfortunately, during sub-maximal exercise arterial O_2 content is usually relatively high and maneuvers which increase the PaO_2 during contractions appear to have minimal impact on the overall level of dilation [148].

A role for hemoglobin was suggested by Ellsworth et al. (1995), involving the release of ATP by erythrocytes depending upon the number of O_2 binding sites occupied [209]. Although there is little direct evidence for this theory it is known that a variety of vasoactive substances including nitric oxide can bind to hemoglobin [210]. This binding appears to be more substantial when hemoglobin is in the oxygenated state so offloading occurs as O_2 is stripped [116, 210].

Conversely, it is proposed that the hemoglobin molecule acts as a scavenger of nitric oxide in the vascular bed, whereby low hemoglobin concentrations would allow greater availability of NO to induce vasodilation [211, 212].

A.4.5. Section Summary

The rapid increase in blood flow accompanying the onset of exercise involves multiple and redundant mechanisms to create a cascade of events to optimize peripheral blood flow and delivery to working tissues. This involves both central cardiovascular upregulation and peripheral adaptations. It appears, at the periphery, that the muscle pump is responsible for the initial increase in blood flow seen at the onset of contractions. Soon afterwards, metabolic dilating substances are released and contribute to the model. Further amplification of this system could be due to flow or ascending metabolic influences on larger proximal arteries. Additionally, any substances transported by the blood may help to coordinate oxygen and blood delivery at a local or systemic level. The influence of dilating substances released by autonomic nerves is currently unclear.

A.5. Circulatory Adjustments to Exercise Training

Having examined the changes brought about by a single bout of acute exercise we must now examine the chronic adaptations the body makes to exercise training. Repeatedly challenging the cardiovascular system to increase the delivery of oxygen and metabolic substrates to, as well as removal of metabolic bi-products and heat from active skeletal muscle results in an increase in maximal oxygen consumption [213]. This increase in $\text{VO}_{2\text{peak}}$ is accompanied by classic adaptations to training in central and peripheral mechanisms including exercise tolerance, skeletal muscle oxidative capacity, cardiac output, cardiac hypertrophy,

bradycardia, and arterial capacitance [39]. More recently other adaptations such as vascular endothelial adaptations to training have emerged. The increased ability of the heart to pump more blood will be outlined in this section, but the focus of the chapter will be the changes in peripheral perfusion to working skeletal muscles.

To provide a solid foundation for considering the training adaptation we must compare changes at maximal and sub-maximal (relative and absolute) VO_2 levels. Although adaptations may be different for short duration, high-intensity activities the focus of this work will be mainly on endurance type activity. Once again the Fick equation provides a scientific base from which to investigate many aspects of cardiovascular adaptations to training. Presented below is a comparison of maximal values for each of the Fick equation components for endurance athletes and sedentary individuals. It can be seen that each maximal oxygen uptake is the product of the three variables to the right. Therefore $\text{VO}_{2\text{peak}}$ is limited by whichever variable cannot increase. Usually, in healthy individuals the stroke volume determines the range over which the heart rate, cardiac output and O_2 uptake can increase [3].

Longitudinal studies supporting the increase in $\text{VO}_{2\text{peak}}$ are also available. The size of the changes depend upon several factors including, the age of the participants, their fitness prior to training, and also the type and length of training undertaken [214]. Generally, for average fitness individuals, $\text{VO}_{2\text{peak}}$ increases by approximately 15-20% following 2 to 3 months of aerobic conditioning [Astrand, 1977 #392]. With continued training greater increases are possible [215].

A.5.1. Central Adaptations

(i) Cardiac Output

When we consider cardiac output is the product of heart rate and stroke volume, it is normal at rest for these variables to act in a reciprocal manner. For example, approximately 5

liters of blood is needed to supply the body at rest. Sedentary individuals have a resting stroke volume of approximately 70ml and therefore the heart must beat 70 times per minute to meet this need. In athletes stroke volume can be as high as 130ml and therefore the heart rate is much lower (40 beats per minute).

Normal values for cardiac output at peak in sedentary individuals is approximately 25 l/min whereas in endurance athletes it can be as high as 42 l/min [216].

(ii) Heart Rate

Normal values for heart rate at peak is similar for both sedentary individuals and athletes at approximately 200 beats per minute. The variation in the variable with training tends to be in the resting heart rate. In sedentary individuals this tends to be approximately 70 beats per minute, whereas in athletes it can be as low as 30 beats per minute [3].

(iii) Stroke Volume

As detailed in table A.1, the increase in peak cardiac output is almost entirely due to the increase in maximal stroke volume. Additionally, at sub-maximal intensities increased stroke volume is the important factor in maintaining cardiac output [214]. The increased range of stroke volume available following conditioning allows heart rate to remain constant at relative intensities of VO_{2peak} .

The current literature is unclear on the exact mechanisms for increased stroke volume. Increases in preload, myocardial contractility, and decreases in afterload all have experimental support. Cross-sectional [217] and longitudinal [218] research has shown increases in end diastolic volume, and wall thickness with training. Although myocardial contractile state increases acutely during exercise it is difficult to match preload and afterload to allow comparisons following training. Blomquist and Saltin suggest any improvements in

Table A.1. Physiological Basis for Differences in $\text{VO}_{2\text{peak}}$

	$\text{VO}_{2\text{peak}}$	=	HR_{peak}	*	SV_{peak}	*	$\text{a-vO}_{2\text{difmax}}$
	(ml/min)		(beats/min)		(ml)		((ml/100ml)/min)
ATHLETE	6250	=	190	*	205	*	16
SEDENTARY	3500	=	195	*	112	*	16

Adapted from [3] p218

contractility, which occur, could be too small to measure [219]. Additionally they suggest that decreased β -adrenergic stimulation due to lower arterial concentrations of norepinephrine influence contractility and heart rate at sub-maximal intensities

A crucial component in the increased stroke volume with training is the ability of an individual to reduce afterload via a reduction in systemic peripheral resistance. Analysis of the performance characteristics of the normal heart suggest that any potential gain in stroke volume that could be achieved by a training-induced increase in heart size would largely be negated by the increased afterload unless training also induced a decrease in systemic resistance [219]. It must also be acknowledged that any improvements in the capacity of the heart to supply blood would be surpassed by the demand of the active tissue when only a fraction of it is utilized. These changes will be address in subsequent sections. For a more detailed review of central adaptations to training see [3, 219].

A.5.2. Peripheral Adaptations

(i) Arteriovenous Oxygen Difference

Estimates attribute half of the increase in $VO_{2\text{peak}}$ to the increase in total oxygen extraction or systemic arteriovenous difference [3]. The effects of increased arterial capacitance and improved delivery of blood flow are responsible for the majority of these changes (this will be addressed in more detail below). The body increases its ability to pump more blood and directs most of this increase to the working tissues. Actual increases in arteriovenous difference per-se are not large. During maximal exercise the oxygen content of arterial blood is approximately 20ml per 100ml of blood and venous content can be as low as 3-4ml per 100ml of blood. This indicates that there is very little oxygen left to be extracted as a result of training [220]. In a longitudinal study Ekblom et al. found 16 weeks of training resulted in a 17%

increase in VO_{2peak} . Arteriovenous difference only increased by 3.6% while cardiac output showed an 8% improvement [221]. Work by Hornig et al. identified low muscle cell partial pressure of oxygen relative to the arterial content is needed to maintain a driving force for diffusion [222]. Since myoglobin facilitated oxygen diffusion mediates a large percentage of total oxygen flux through the sarcoplasm it is interesting that there is no increase in myoglobin concentration with exercise training in humans [223], indicating it is probably not a limiting factor in oxygen flux.

An increase in the efficiency and volume of oxidative muscle cell organelles and enzymes occur with training and help to increase the diffusion gradient for oxygen from the blood. The mitochondria are the sites in the muscle cell where oxygen is consumed. Unfortunately, there is only a modest increase in VO_{2peak} when mitochondrial enzymes are increased more than 2-fold [224]. The nature of these adaptations are beyond the scope of this review but include, an increase in the number and size of mitochondria (and constituent enzymes such as cytochrome c) [225], an increase in lactate dehydrogenase [226], along with a relative decrease in glycolytic flux [227]. The main effect of increasing mitochondrial enzymes is to improve endurance performance by greater utilization of fats as fuels and less production of lactate [228]. For a more detailed review of this area please refer to references [225, 229].

The strongest relationship between training-induced adaptations and increases in whole body maximal aerobic power is the increase in the ratio of capillaries per muscle fiber [225]. These adaptations are once more related to delivery and allow greater transit time of oxygen through the capillaries and in turn greater diffusion to myofibrillar mitochondria through the sarcoplasm [230].

A.5.3. General Redistribution of Blood Flow

A.5.3.1. Rest and Pre-exercise

Exercise training doesn't appear to have any effect on blood flow during resting conditions [129]. However, an anticipatory response is evident and research suggests that blood flow is increased proportion to the work about to be performed. Suggestion of higher intensity work leads to higher cardiac outputs, arterial pressures, and limb blood flows, than for the suggestion of lower intensity work [134]. It also appears that the intensity of training can influence the distribution of blood flow among muscles during pre-exercise, with low intensity long duration training elevating flow to Type IIa fibers and high intensity training elevating flow to both Type IIa and IIb fibers [129, 231]. Even at this anticipatory stage of exercise, specificity of training is evident. The elevation of blood flow depends upon if that muscle was recruited during the proceeding training sessions [156]. It is unclear what factors contribute to this response. In some animal models sympathetic cholinergic vasodilation may occur [134]. In humans, evidence of this is negligible, but a decrease in arteriole sensitivity to sympathetic nervous activity or an increase in endothelial-mediated dilation may be responsible [232, 233].

A.5.3.2. Sub-Maximal Exercise

During sub-maximal exercise there is no difference in whole body VO_2 or cardiac output with training [111, 213]. Following exercise training, any relative $\text{VO}_{2\text{peak}}$ intensity will be reached at a higher absolute workload. Therefore, comparisons between pre and post training are more appropriate when based on absolute exercise intensity.

The previous chapter detailed the magnitude of reductions in blood flow to renal and splanchnic regions and showed they are directly related to the relative exercise intensity. In 1964, Rowell et al. found at exercise at an absolute work level equivalent to 60% $\text{VO}_{2\text{peak}}$ there

was a 40% fall in hepatic blood flow. Following endurance training the same workload (now equal to 50% $\text{VO}_{2\text{peak}}$) caused only a 30% fall in hepatic blood flow. In highly trained endurance athletes this work load (45% $\text{VO}_{2\text{peak}}$) elicited a 25% fall in hepatic blood flow [234]. Clausen et al. confirmed this adaptation but showed it was specific to only the muscles which had undergone training. Their subjects underwent arm or leg training. Hepatic blood flow was higher only when the same absolute exercise was performed in the trained limb and was no different when untrained limbs were used [235]. These changes appear to be caused primarily by lower plasma norepinephrine concentrations and possibly by reduced sensitivity of the tissues to norepinephrine [236].

In humans, studies suggest working muscle blood flow is unchanged [237, 238] or even slightly reduced [239, 240]. However, as in pre-exercise anticipation, the distribution of this flow is different. Endurance training leads to increased perfusion of primarily high oxidative fibers [129], and diminished perfusion of low oxidative fibers [231]. Factors responsible for muscle blood flow redistribution are many-fold including, an increase in motor unit recruitment, an increase in the amount or type of metabolic vasodilator/s released, decreases in the amount of sympathetic stimulation to specific fibers or a decrease in sensitivity to norepinephrine, and/or enhanced endothelial dependent dilation of the microvessels [213].

A.5.3.3. Maximal Exercise

Since approximately 85% of cardiac output is directed to the working skeletal muscle during exercise at $\text{VO}_{2\text{peak}}$ [155, 241] and cardiac output is increased with training, then accordingly the working muscles should receive a relative increase in flow [242]. Crucial to this supply is the ability of the heart to maintain arterial pressure during the dramatic increase in vascular capacitance in the working tissues. Therefore, a dynamic balance is created whereby

when the working tissue is small, cardiac output can meet the increased blood flow requirements and maintain arterial pressure. However, when a critical mass of exercising muscle is reached the defense of arterial pressure takes precedence. For example peak blood flow of oxidative muscle can often approach 300-400ml/min per 100g. Therefore if a normal 75kg human has approximately 30kg of muscle and exercises only 50% of that mass, cardiac output would have to be in the range of 45-60L/min (15000g muscle * 300-400ml blood) [243]. This principle is confirmed in classic studies by Davis and Sargeant (1975), and Klausen et al. (1982). Both groups trained both legs of subjects, one leg at a time. Davis and Sargeant observed peak oxygen uptake during “maximal” one-legged exercise was approximately 0.35l/min in each leg, whereas the increase in $\text{VO}_{2\text{peak}}$ in two-legged exercise was only 0.14l/min [244]. Klausen’s group explained this with their finding that blood flow was similarly reduced in each leg when they were exercised together compared with alone [245].

A.5.3.3.1. Cardiac Output vs. Vascular Capacitance

This balance between muscle capacitance and cardiac output forms the crux of the debate about the limitations to aerobic exercise performance. Fortunately, in theory, we have some absolute parameters with which to work. Cardiac output falls within a known parameter, approximately 25l/min for most individuals, of which possibly 21l (85%) is available to supply muscle. These figures set the upper limit for total muscle blood flow. Therefore, if all of the 30kg of muscle were employed peak blood flow would be approximately 70ml/min per 100g of tissue. Even in an endurance trained athlete with a maximal cardiac output of 42l/min peak blood flow would be approximately 109ml/min per 100g of tissue. Clearly this is well below the 300-400ml/min per 100g previously observed. Along these lines to achieve a muscle blood flow

of 300ml/min per 100g only 7kg of muscle mass, or 25% of total skeletal muscle mass could be perfused.

It therefore becomes apparent that the greater the muscle mass we activate, the less blood flow can be supplied per 100g of tissue. Additionally, to reach an upper limit for muscle blood flow we must maximally activate a relatively small muscle mass so not to outstrip the pumping capacity of the heart [243].

Unfortunately, maximal muscle blood flow can never be obtained in an intact setting. In fact it could only be measured on a small muscle mass at a specific arterial pressure and vascular conductance. Given that...

$$Q \propto \text{MAP} * \text{TPR}^{-1}$$

...any change in cardiac output is countered by adjustments in total peripheral resistance (or capacitance in exercise). Additionally, add to the complexity of this model, any decreases in working skeletal muscle vascular resistance is often counteracted by increases in non-working skeletal muscle vascular resistance to help maintain arterial pressure. This is even more evident in maximal exercise when cardiac output is approaching its capacity. The coordination of vascular resistances to optimize blood flow to working tissue and maintain arterial pressure occurs primarily due to the ability of local influences to attenuate the vasoconstrictive influence of the sympathetic nervous system.

A.5.3.3.1. Capillary Density Changes

Increases in muscular perfusion at $\text{VO}_{2\text{peak}}$ appear to be as a result of increased capillary density, dilation of more arterioles associated with the recruitment of more motor units, dilation of newly formed arterioles, greater dilation of existing arterioles, and vascular remodeling (increased length, cross-sectional area and/or diameter of existing arteries and veins) [213].

These changes are specific to the muscle tissue recruited during training [246, 247]. But, unlike animal studies, human training-induced increases in capillarity appear to be more uniform in terms of fiber type [248, 249].

The significance of greater capillarization on active muscle blood flow is the idea that the number of resistance arterioles may also be increased [247, 250]. Secondly, the muscle pump may exert a greater effect because it could expel and in turn draw a greater volume of blood from/through [213]. Alterations in the vasodilatory properties of individual arteries and arterioles could also allow for greater vessel radius. Mechanisms for this are speculative, but could include changes in vessel responsiveness to neurohumoral stimuli, modification of myogenic responsiveness, and changes in metabolite release/sensitivity.

A.5.3.3.3. Neurohumoral Changes

Plasma norepinephrine levels following training may actually be higher at VO_{2peak} than in sedentary individuals [251], but these gross measures do not appear to reflect specific muscle sympathetic nerve activity examined using microneurography [252].

Several studies have indicated decreases in sympathetic outflow to trained muscle and/or attenuated muscle responsiveness [253-255]. Somers et al. (1992), reported that the increases in muscle sympathetic nervous activity during 2 minutes of 33% MVC isometric handgrip exercise decreased from 111% prior to training to 38% following training in the conditioned arm, as opposed to no change in the untrained arm. This decrease was attributed to decreased activation of the muscle metaboreflex [255]. Sinoway found training specific reductions in muscle sympathetic nerve activity were specific to the limb trained and attributed this to a change in the muscle mechanoreflex [254]. Finally, Sheldahl et al, (1994), found no modification of sympathetic response during isometric forearm exercise in subjects that underwent 12 weeks of

leg training [256]. These studies show exercise-induced increases in muscle sympathetic nerve activity are attenuated following training. These changes are specific to the trained muscles and may play an important role in blood flow adaptations to training [252].

Other changes to the neurohumoral control following training include a change in the number of adrenergic receptors [257]. Interestingly, evidence suggests that the vasculature of muscles comprised of primarily type I fibers are much less influenced by sympathetic adrenergic stimulation than muscles comprised of primarily type IIb fibers [258]. Therefore any conversion of ‘fast’ fibers to ‘slow’ fibers by exercise training may influence the blood flow responsiveness to an acute exercise bout.

A.5.3.3.4. Endothelial Control Changes

The endothelial contribution to increased arterial capacitance with exercise training appears to be threefold; to contribute to the reduction in vasoconstrictor reactivity, to increase vasodilator reactivity, and to contribute to vascular remodeling [177].

Endothelial cells have been shown to exhibit α_2 -adrenergic receptors which mediate the release of nitric oxide in the presence of norepinephrine [259]. This was demonstrated by Delp et al. in endurance trained normal [232] and hyperthyroid rat [260] aortic vessel rings. The study was conducted in a medium bath containing propranolol to negate a β_2 -receptor effect. However, the removal of the endothelial cell layer abolished the reduced sensitivity to norepinephrine in the vessel rings from the trained rats [232], and the phenylephrine response (an α_1 -adrenergic agonist) was not altered by training. Additionally, the endothelium may reduce vasoconstrictor responsiveness by attenuating the release of norepinephrine from adrenergic nerves [261].

The first work showing increased vasodilator reactivity was performed by Miller and colleagues [262, 263]. They demonstrated that canine femoral arteries exposed to chronic increases in blood flow, via opening an arteriovenous anastomosis, exhibited enhanced endothelium-dependent relaxation in vitro. In 1992, Lash and Bohlen, investigated functional adaptations of arterioles within the skeletal muscle of rats aerobically trained for 8-10 weeks with treadmill exercise. They found large and intermediate sized arterioles of the spinotrapezius dilated proportionally more in the trained group to 1-8Hz stimulation [264]. Similar responses to exercise training have been found in various tissues such as coronary arteries [265-268], thoracic and pulmonary arteries and arterioles [269], the abdominal aorta [53, 232, 260] and skeletal muscle tissues [233, 264, 270-272]. The issues surrounding this research concern the length of time needed to cause endothelial modifications to the vasculature, the caliber of vessels involved and the nature of the etiology of the modifications.

Most aerobic training studies in the literature were designed to last between 8-10 weeks [177, 232, 260, 264, 269, 273], although Delp and associates designed a protocol to examine the time-course of enhanced endothelium-mediated dilation in the aorta of trained rats [53]. They treadmill ran groups of rats for 1, 2, 4, and 10 weeks periods at 30m/min on a 15% incline for 1hr/day, for 5days/week. The rats were tested with 24hrs of their last exercise bout. Citrate synthase activity was significantly elevated, in comparison to controls, after 2 weeks (15%), and continued to increase to after 4 weeks (26%) and 10 weeks (33%) training, demonstrating a training stimulus sufficient to enhance skeletal muscle oxidative capacity. To measure vasodilation, the aortic segments were first treated with norepinephrine, which induced a similar tension in all groups. A single dose of acetylcholine was then used to induce endothelial-mediated vasodilation. The percent dilation in sedentary rats (61%) was similar to that in ring

segments from animals in the 1 day, 1 week and 2 week training groups. However, the percent relaxation of the vessel rings was greater in the 4 week (72%) and 10 week (79%) trained rats. Acetylcholine did not change norepinephrine-induced tension in vessels denuded of the endothelial cell layer. Interestingly, blockade of cyclooxygenase, and therefore prostaglandin production, with idomethacin in intact vessels, did not alter the acetylcholine response. However, treatment with the nitric oxide synthase blocker, L-NAME abolished the vasodilatory response. Additionally, the amounts of endothelial cell nitric oxide synthase protein were greater after 4 weeks training. These findings lead to the conclusions that responses to the endothelium-dependent dilator ACh are enhanced by 4 weeks of endurance exercise training, and this enhancement is preceded by increases in skeletal muscle oxidative capacity. In addition, the elevation appears to be mediated exclusively through the nitric oxide synthase-EDNO pathway in the rat aorta and is associated with increases in the expression of eNOS protein [53].

Delp's findings are in agreement with those of Muller et al. (1994), in coronary resistance vessels responses to bradykinin, idomethacin and L-NAME after 16-20 weeks of training [265] and those of Sun et al, (1994), in the arterioles of the rat gracilis muscle following 2-4 weeks treadmill training [233]. A plethora of studies also support nitric oxide mediated dilation following training [53, 177, 232, 266, 268, 270, 272, 274-276]. Taken together these findings indicate an up-regulation of endothelial cell nitric oxide production with training. This process involves increases in mRNA [266, 276-278] and associated endothelial derived relaxing factor proteins [266, 274, 276].

Interestingly, some studies have also shown that endothelium-dependent flow-mediated dilation in rat gracilis arterioles is regulated by the corelease of nitric oxide and prostaglandins and that they act in an additive manner [275, 279]. Furthermore, in a subsequent experiment

exercise training enhanced this response by 83.5% at maximum flow. This enhanced sensitivity maintained a lower shear stress in the exercised rat's arterioles. Finally, inhibition of each of the dilator pathways reduced flow-dependent dilation to a similar degree (approximately 40-45%), and their combined inhibition nearly completely eliminated the dilation of arterioles of both sedentary and exercise trained rats [272]. Although interesting, it should be noted these findings all originate from the same laboratory. Additionally, this study utilized shear stress as the in vitro stimulus rather than a pharmacological stimulus to initiate dilation. This could possibly indicate different receptor/mechanisms are activated with different stimuli. Currently, the literature has not provided a sufficient data to address these issues.

This upregulation of vascular endothelial responsiveness may be a temporary adaptation which acts as a stimulus for vascular remodeling [39]. Laughlin investigated the role of endothelium derived relaxing factors in the control of coronary vascular resistance in subjects who have been exposed to exercise training for a period of time sufficient to complete the adaptation process. He found in conduit coronary arteries endothelium-mediated vasodilator mechanisms are enhanced during the initial phases of exercise training but return to normal by the time the animals have completed the adaptation to exercise training [39]. The coronary microcirculation, however, appears to maintain enhanced responsiveness. He concludes that structural adaptations may occur as part of the exercise adaptation which produce a relative decrease in coronary shear during exercise [39]. Again these results are interesting but must be viewed in context. There are currently no studies supporting this hypothesis in human coronary vessels or in fact in the peripheral vasculature. Additionally, non-cardiac vessel time course investigations currently do not support this idea [53].

A third function of increased endothelial function in peripheral arteries is its influence on vascular remodeling. Because both growth-promoting and growth-inhibitory factors are made and secreted by endothelial cells, they are pivotal in the control of vascular responsiveness, particularly in the case of abnormal growth of smooth muscle during disease [24]. Accordingly, this topic will be addressed in more detail in chapter 5. Nevertheless, it is noteworthy here that, modifications to vessel caliber have been noted following exercise training. Laughlin and co-workers noted structural remodeling of canine coronary arteries to become larger in diameter which occurred as exercise training continued beyond 1 week [38]. He hypothesized that this may occur to reduce shear stress on the vessel walls.

In humans studies involving small muscle groups have shown training induced improvements in peripheral vasodilation occur in the absence of central hemodynamic and sympathetic modulations [280-282]. Research identifying endothelial derived relaxing factors as responsible for these increase in flow have been conflicting. Green et al, found increases in forearm hyperemic blood flow and decreases in vascular resistance following 4 weeks of handgrip training but failed to find any augmentation of the dilatory response to methacholine chloride or sodium nitroprusside [281]. In contrast Kingwell et al. reported enhanced dilation to acetylcholine in the forearm of endurance trained athletes compared to sedentary controls, with no difference in the response to sodium nitroprusside [283]. In 1993, Katz et al. showed that 8 weeks of lower extremity aerobic training was associated with a specific increase in acetylcholine-mediated dilation in the trained lower extremity but not in the untrained upper extremity in normal subjects [284]. The differences in these studies could be related to the intensity of the exercise stimulus. Green et al. had subjects train for 30 min, 4 times per week (for 4 weeks) at an intensity of approximately 70% of a resistance that could be maintained for 3

min [281]. Most recently, Clarkson et al. examined the effects of a 10 week aerobic and anaerobic (Army basic training) exercise program on flow-mediated and nitroglycerine-induced vasodilation in 25 healthy male military recruits. Flow mediated dilation increased from 2.2% to 3.9% following training ($p=0.01$) with no difference in nitroglycerine mediated dilation [285]. Interestingly, these findings are supported by localized training protocols implemented in heart failure patients [286, 287].

It is interesting that when examining the endothelial response to exercise training pharmacological approaches have provided conflicting results, whereas flow mediated dilation has consistently reported increases in vasoresponsiveness. This could be due to varying methodological approaches, and in fact Joyner et al. suggest that infusions given via the brachial artery in humans may in fact reflect events at the resistance arteries rather than the conduit vessels [179]. When this is considered in conjunction with Laughlin's schema of nonuniform vasomotor responsiveness of the arterial tree, albeit in the coronary vasculature [38, 39], it is possible that different locations or receptors/pathways are measured. This could be a significant distinction because the in ascending theory of vasodilation attributes the microvessels for initiating the increase in blood flow and the conduit vessels adjust the magnitude of flow [51, 204].

A.6. Effects of Disease on Peripheral Circulation and Adaptation to Exercise

Viewing exercise physiology from a clinical perspective demands an examination of the impact of cardiovascular risk factors and disease states on vascular control mechanisms.

Traditionally, there appear to be two major schools of thought within the literature. The larger of these involves endothelial function and its involvement in cardiovascular disease pathogenesis.

The second school of thought involves autonomic dysfunction and its impact on disease. Unfortunately, most of the findings from either ideology take on a univariate approach and therefore attribute vascular dysfunction to the action of the particular variable of consideration whilst, failing to provide a combined model for disease initiation, progression, and possible interventions. A third emerging school of thought is apoptosis and involves a form of actively regulated cell death. The focus of this section is to describe the changes in the peripheral vasculature accompanying and/or promoting the disease process. This review will focus on peripheral arteries and only reference systemic changes briefly as they impact the vasculature.

Cardiovascular disease is the major cause of disease globally and accounts for approximately 20% of all mortality [288]. In the United States in 1995, approximately 54% (960,000) died of cardiovascular disease and stroke [289]. The current thinking in the pathogenesis of atherosclerosis proposes that “injury” to the intima or endothelium is the initiating event [290]. The progression of the disease process and its effects on vessel structure is relatively well established but is worthy of brief review.

A.6.1. The Pathogenesis of Atherosclerosis

Atherosclerosis is defined by the World Health Organization as...

“a variable combination of changes of the intima of arteries consisting of the focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits...”

The earliest lesion of atherosclerosis is known as a fatty streak and contains lipids-laden macrophages and underneath these are lipid-laden smooth muscle cells [291]. Macrophage cells play an important role in the development of the atheroma. They promote an inflammatory response to injury and in combination with lipids create an active “foam cell” environment prone to rupture and further inflammatory debris accumulation. These sites progress to fibro-muscular

lesions, which possess a dense hardened, fibrous cap of smooth muscle and connective tissue. The fibrous cap of these lesions contain multiple layers of a special form of smooth muscle cell, each of which lies in a lacunae-like arrangement in which the lacunae consist of alternating layers of basement membrane and proteoglycan. Beneath this cap, the lesions are highly cellular and contain smooth muscle and macrophages, which may contain lipid droplets surrounded by connective tissue. Other leukocytes may also be present in these lesions. Beneath this there may be an area of necrotic debris, cholesterol crystals, and calcification [290]. The increased proliferation of smooth muscle cells pushes the “plaque” into the lumen of the vessel creating a rigid narrowing of the artery.

The key questions for this review involve the development of the circumstances that contribute to the initial intimal damage and the progression of the vessel atheroma. Epidemiological studies have produced a multitude of risk factors for cardiovascular disease development [292-296] and although the exact mechanism of lesion progression may never be totally clear we have a good scientific understanding of how these risk factors influence vascular health. Physical and chemical stresses have been shown to induce intimal damage and vascular dysfunction. Although, currently it is not clear which comes first. Does the vessel become less responsive which, predisposes the lining damage or, does damage occur first and cause the vessel to be less responsive?

A.6.2. Apoptosis

Apoptosis is a form of actively regulated cell death. Various stimuli, using a variety of receptors and signal transduction pathways, activate a cascade of proteases that execute a program of cellular self-destruction by cleaving cellular structures at specific sites in a strictly controlled manner [297]. This occurs without cell content spillage and inflammatory responses.

(For a full review of the apoptotic process please refer to [298, 299]). This process has recently been linked with several cardiovascular risk factors and with vascular disease progression itself. The influence of apoptosis on vascular atherogenesis centers on three major concepts; apoptotic influences cause endothelial damage/dysregulation, upregulate smooth muscle turnover and growth, and increase macrophage proliferation at the site of intimal lesions. Although a majority of studies in this area are correlational, and cause and effect is yet to be established, the weight of numbers of studies warrants a brief synopsis.

Endothelial cell apoptosis has been shown in atherosclerosis [300], hypertension [301], and congestive heart failure [302]. Yet, apoptotic endothelial cells are rarely found in the intima of vessels that show no evidence of vascular disease [303]. Although there is evidence that endothelial apoptosis is present in vessel areas with low shear stress prior to disease manifestation [304], it is currently unclear if apoptosis is responsible for the initiation of disease or if the disease process causes cells to become apoptotic.

Changes associated with endothelial apoptosis are embedded in a complex array of interdependent events [297]. Apoptotic endothelial cells have been shown to release the vasoconstrictor interleukin-1 [305]. In turn, this enhances apoptosis, endocytosis of apoptotic bodies and can activate apoptosis in neighboring endothelial cells [305, 306]. Interleukin-1 is also associated with the expression of adhesion molecules for inflammatory cells, leading to leukocyte adhesion and increased presence of oxidized-LDL's [307], which happen to be extremely apoptotic bodies. An increase in the apoptotic tumor necrosis factor is also involved in this cascade.

Normal anti-coagulant endothelial properties are lost during apoptosis [308]. For example PGI₂ production is decreased [309]. This particular area of apoptosis and inflammatory

responses is a little confusing at the moment with reports of pro and anti-apoptotic effects. This confusion maybe related to attempts of to limit the inflammatory process to a particular area of damage. For example, monocytes can release the pro-apoptotic TNF but, when monocytes are indirect contact with endothelial cells they prevent apoptosis in a pro-apoptotic environment [310].

Unlike the endothelial destruction, vascular smooth muscle proliferation and hypertrophy are associated with atherosclerotic and hypertensive disease states [290]. In vascular tissue the balance between cell proliferation and apoptosis determine the extent of smooth muscle growth [311]. In atherogenesis apoptosis is decreased and the homeostasis is interrupted in favor of growth. For example PDGF, angiotensin II, and thrombin promote H_2O_2 production in the smooth muscle [312] which, in turn, activates p38 MAPK which is implicated in hypertrophy [313]. Again, this research area is confusing because it is also apparent that some radical oxygen species are essential for the apoptosis of vascular smooth muscle [314].

In dynamic plaques of pre-existing lesions formed in atherosclerosis apoptosis is promoted by NO and promotes plaque reductions and macrophage destruction [315]. In fact, Wang and colleagues fed male New Zealand white rabbits a high cholesterol diet for 12 weeks and added 2.5% L-arginine in the drinking water for the final two weeks. They found L-arginine treatment increased the number of apoptotic cells (largely macrophages) in the atherosclerotic lesions by 3-fold [316]. In subsequent in vitro experiments, sodium nitroprusside or L-arginine caused a similar effect. These results were linked to an increase in the nitrogen oxides released into the medium.

The current difficulty with this field of research is its modernity and the heterogeneity of the current findings. Most of the research in this area is post 1997 and consistent patterns of

findings although apparent appear to vary between tissues, types, vessel locations, species and, duration and type of exposures used [297]. Therefore, although it cannot be considered proven at this time, the available data points toward involvement of endothelial, vascular smooth muscle and, lesion apoptosis in the progression and regression of vascular disease. To strengthen this argument, known pro- and anti-apoptotic agents need to be evaluated, under various conditions, as treatment strategies for vascular diseases.

A.6.3. Autonomic Dysfunction

The autonomic nervous system has a major influence over cardiac and vascular function and alterations in autonomic function have important physiological, pathophysiological and clinical implications [317]. A good model for the demonstration of the influence of the autonomic nervous system on cardiovascular disease is essential hypertension. Essential hypertension is defined as high blood pressure for which no specific cause can be found [318], and accounts for 95% of all hypertension cases [319]. Hypertension is also the most pervasive form of physical stress on the vessel intima and could contribute to endothelial damage. In young, borderline hypertensives vascular resistance appears to be normal but pressure is elevated by an increased cardiac output [320]. This condition is characterized by both increased sympathetic drive and decreased parasympathetic restraint because both propranolol and atropine combined are needed to abolish the difference between borderline hypertensive and normal subjects [321]. These findings point towards a central modification of blood pressure regulation rather than a change in receptor sensitivity [320]. In this review our interest is mainly in the autonomic control of vascular resistance, which appears to be similar between normotensive and borderline hypertensives in many cases. In fact, in normotensive individuals any increase in cardiac output is compensated for by a decrease in vascular resistance, which allows

normalization of blood pressure. Borderline hyperkinetic hypertensives do not make this adjustment and tend to demonstrate widespread plasma catecholamine spillover [322]. It appears they have a relatively normal response to stress including, isometric and dynamic exercise [323], blood volume expansion [324], and cold pressor tests [325]. This leads to the conclusion that blood pressure regulation around the set point is normal but that set point has been raised.

Borderline hypertension is a progressive disease and often develops in to essential hypertension. This disease state is characterized by increased sympathetic outflow in young subjects. These observations have been made using microneurography recordings of nerves supplying skeletal muscle [326, 327], noradrenaline spillover in cardiac and renal beds [328]. Additionally, heart rate variability techniques show the low-frequency component is depressed and the high-frequency component is elevated compared with normotensive individuals [329], suggesting enhanced sympathetic and reduced vagal activity. Differences between normotensive and essential hypertensive subjects are less evident with increasing age [330]. In fact, in older primary hypertensives plasma norepinephrine levels may in fact be lower than normotensives [331]. Interestingly, spectral analysis of heart rate variability data has revealed vagal tone may still remain depressed [332]. It is not clear what controls this transformation from a high output to a high resistance state but it is believed changes in vessel caliber due to injury and possibly endothelial dysfunction may elevate blood pressure and reduce the reliance upon cardiac output to meet an elevated baroreceptor set point [320]. Additionally, the effect of increased resistance is to alter the perfusion of the kidneys and initiate an upregulation of the renin, angiotensin and aldosterone cascades with the resultant increase in plasma volume and a further reduction in vascular conductance. Eventually, as this condition continues constant vascular afterload causes a left ventricular overload and depression of cardiac output.

This section is not designed to provide a complete overview of hypertension or of autonomic arterial pressure regulation, but to recognize its role in disease etiology. To this end, research has linked autonomic dysfunction with established risk factors for cardiovascular disease [333, 334], markers of cardiovascular disease itself [335-337], diabetes mellitus [338, 339] and, aging [334, 335, 340]. Therefore, the importance of autonomic function on the peripheral vasculature and in health and disease is acknowledged and thus allows the proceeding work to be considered within a wider context of vascular control.

A.6.4. Endothelial Dysfunction

In 1980, Furchgott and Zawadzki discovered the importance of the endothelial cell in the process of vascular relaxation/dilation in rabbit aorta [25]. Since that study research in this area has progressed rapidly. Animal and human studies have consistently demonstrated endothelium-dependent relaxation of the vasculature is impaired in disease models. Our current understanding of this area implicates the endothelium in the progression or initiation of certain diseases and their clinical complications [37, 189].

The role of the endothelium is not limited to the ability to regulate vascular tone and vasodilate vessels, but also includes providing a physical barrier to protect medial smooth muscle, maintaining hemodynamic homeostasis, and the inhibition of smooth muscle cell proliferation and migration. These functions are mediated by the synthesis and release of a variety of substances including vasodilators such as nitric oxide and prostacyclin, vasoconstrictors such as endothelin and thromboxane, anticoagulants such as tissue plasmin activator and antithrombin III and procoagulants such as von Willebrand factor and plasminogen-activator inhibitor. During endothelial cell inhibition or damage the normal homeostatic balance becomes disrupted in favor of an atherogenic environment.

This section will attempt to outline the roles and mechanisms of endothelial function in vascular health and disease, along with factors known to damage or disrupt endothelial function and the implications for vascular health. The subheadings adopted try to sectionalize the roles but considerable overlap occurs.

A.6.4.1. The Roles of the Endothelium

A.6.4.1.1. Provide a Physical Barrier

This is the most obvious contribution of the luminal layer of the vasculature. A normal endothelium is a layer of cells with tight intercellular junctions. Despite only being one cell thick, this layer is able to “sense” changes in hemodynamic forces, or blood-borne signals, and respond to physical and chemical stimuli [26]. Given that shear stress on the arterial endothelium can range between 10 and 70 dyne/cm², the endothelium acts to reduce these forces on the medial layer [40]. Under “normal” conditions shear appears to be approximately 15 to 20 dyne/cm² [41, 341]. In fact, chronic increases in shear stress in models such as the radial artery of dialysis patients proximal to arteriovenous fistula lead to an expansion of the luminal radius such that mean shear stress is returned to its baseline level [341], in an endothelium dependent process [41].

Two theories of shear stress and plaque localization exist. Initially, it was believed high stress (400 dyne/cm²) directly damaged the endothelial layer and provided access to the vessel medial layer [342]. More recently, low shear (± 4 dyne/cm²) has been implicated in the atherosclerotic process [343, 344]. This is supported by the formation of plaques at the outer walls of bifurcation's and areas of blood flow recirculation and stasis [40]. Low shear has been implicated with a decrease in endothelial cell proliferation and consequent cell loss, desquamation, altered morphology with decreased elongation, decrease in actin stress fibers,

greater monocyte attachment and migration across the endothelial layer and increased expression of vascular cell adhesion molecule-1 (VCAM-1) [345]. Apoptosis may also play a role in endothelial cell loss [346].

The second to second variations in shear stress appears to have a beneficial effect on the endothelial barrier, with increased secretion of nitric oxide and prostacyclin and the subsequent decrease in platelet activation, smooth muscle proliferation and neointima formation [347]. Turbulent flow fails to induce alterations in endothelial cell calcium concentrations and activate protein kinases [348]. It also allows the upregulation of endothelin-1 mRNA [349] and increases the presence of superoxide anion [350], which are both atherogenic. It is established that chronic hypertension injures the endothelium but it is unclear as to whether this is related to the constant pressure or to an increase in vessel turbulence. Regardless, the physical stress associated with chronic high flow damages the endothelial barrier, exposing the medial layer, triggering an inflammatory response and contributing to a pro-thrombotic environment.

A.6.4.1.2. Regulate Vascular Tone and Vasodilate Vessels

Regulation of vascular tone is important for not only maintenance of optimal shear stress. It is also implicated in the control of blood flow to working tissues [188, 199-202]. Under clinical conditions this becomes increasingly significant in maintenance of normal tissue perfusion.

The endothelium can release various factors to dilate or constrict vascular smooth muscle. Nitric Oxide is the most documented vasodilator and its release can be triggered by platelet derived factors such as serotonin, histamine, fatty acids; or hormones such as acetylcholine, norepinephrine, bradykinin, vasopressin and thrombin; or by some metabolites such as adenosine diphosphate [189]. Other vasodilators released by the endothelium include

prostaglandin and endothelial-derived hyperpolarizing factor (see section 4 for details of chemical release pathways).

The endothelium also releases vasoconstrictor substances such as endothelin, platelet derived growth factor (PDGF), thromboxaneA₂, active oxygen species. Endothelin-1 has received the most focus of the endothelial-vasoconstrictors. An enzyme known as endothelin converting enzyme (ECE) is involved in endothelin production. Interestingly, mRNA for ECE is stimulated by thrombin, transforming growth factor- β , interleukins, epinephrine, angiotensin II and vasopressin [351]. Endothelin-1 is a complex molecule which at lower concentrations evokes vasodilation and at higher concentrations evokes sustained and intense vasoconstriction [352]. This property is an interesting example of endothelial control. Endothelin-1 production is inhibited by a cGMP dependent mechanism [353], by a cAMP mechanism, and by an unidentified product produced by vascular smooth muscle [354]. Blocking the L-arginine pathway, whereby thrombin- or angiotensin-induced endothelin production increases, can alter the balance of this mechanism in favor of vasoconstriction. Interestingly, endothelin-1 causes the release of NO and PGI₂ from endothelial cells in a negative feedback mechanism to limit the production of Endothelin-1 and vasoconstriction. Therefore, at low levels endothelin-1 induces dilation via NO and PGI₂ whereas, at higher concentrations it has a dominant effect.

Control and counter-control mechanisms are a feature of many physiologic homeostatic systems. In these systems, as with the endothelium, damage or prolonged perturbations often result in dysfunction and an unhealthy environment prevails. When the endothelium becomes dysfunctional it loses its vasodilating mechanisms and the underlying smooth muscle becomes sensitive to vasoconstricting substances. In fact, a loss of coronary artery vasodilatory capacity to Ach has been shown prior to overt atherosclerotic lesions in humans [355]. Additionally,

vasoconstriction may occur under conditions in which vasodilation would normally be present [189]. For example, the normal vasodilatory response of isolated artery to acetylcholine is replaced by paradoxical vasoconstriction [Ludmer, 1986 #507].

Additionally, tonic NO production may be inhibited by damage. Given that basal circulation is in a constant state vasodilation and tonic NO secretion it is no surprise that in isolated arteries, L-NAME or other NO inhibitors induce endothelium-dependent contractions [356]. Additionally, infusing NO inhibitors into the human forearm also results in decreased forearm circulation [44]. In fact endothelial dysfunction has been implicated in essential hypertension [357, 358] and nitric oxide deficient animals have a markedly reduced survival rates due to target-organ damage suggests NO exerts an important protective role in the circulation [351].

A.6.4.1.2. Maintaining Hemodynamic Homeostasis

An intact endothelium presents an anticoagulant surface due to the action release of NO, PGI₂, antithrombin III, thrombomodulin, herprin-like substances, tPA, and superoxide dismutase. Additionally NO and PGI₂ inhibit platelet aggregation and the secretion of platelet contents [359, 360]. Thrombin, a focal part of the thrombosis cascade, is cleared by endothelial released antithrombin III and thrombomodulin [361]. In fact, key signals to stimulate the endothelial-dependent production of NO and PGI₂ are thrombin [362, 363], and platelet released ADP and ATP [364]. Therefore, at sites where platelets and the coagulation cascade are activated, intact endothelial cells release NO and PGI₂, which, in turn, initiates vasodilation and platelet inhibition, thereby preventing vasoconstriction and thrombus formation [351].

When the endothelial cell becomes dysfunctional there is a local accumulation of excess active oxygen species, inflammatory cytokines and abnormal recruitment of monocytes and

lymphocytes into the vessel wall. These substances may shift the normal anticoagulant milieu to one of thrombosis. A dysfunctional endothelium also releases platelet-activating factor and platelet derived growth factor which enhance the pro-coagulant environment [189], and thromboxane A₂, a potent vasoconstrictor, causing vasospasm, thrombus formation and vascular occlusion [31].

A.6.4.1.4. Inhibit Smooth Muscle Cell Proliferation and Migration

The normal endothelial cell is thought to prevent vascular smooth muscle cell proliferation via production of NO, PGI₂, and heparin-like substances. The increase in smooth muscle cGMP levels accompanying NO stimulation has been found to be growth inhibitory, although the mechanism is unclear [365]. Additionally, the PGI₂ induced increase in cAMP concentration appears to have an inhibitory effect on smooth muscle growth. Endothelial cells may be involved in the action of other growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor, angiotensin II and endothelin-1. For example, endothelial released transforming growth factor- β (TGF- β) is activated by smooth muscle and inhibits growth directly at the site and indirectly by inhibits PDGF secretion and extracellular matrix composition.

A.6.5. The Relationship Between the Endothelium and Risk Factors for Cardiovascular Disease

Endothelial dysfunction may result from several forms of injury, such as oxidative stress, advanced glycosylation end products, hemodynamic stresses, inflammation, and oxidized lipids [189]. There is an extensive body of recent research in this area linking these mechanisms with a variety of cardiovascular risk factors and/or conditions ranging from hypercholesterolemia [366, 367], and hypertension [368, 369], to diabetes [370-372] and heart failure [373] models. All these processes involve disruption or destruction of the normal endothelial homeostatic balance

and often degradation of the NO system and a consequent inability to vasodilate. In animal models, relatively short-term in vivo exposure to cardiovascular risk factors such as lipids [374, 375], glucose [376] or hypertension [377] results in endothelial vasomotor dysfunction, alteration in endothelial adhesion and infiltration of circulating inflammatory cells, and neointimal growth which are similar to changes observed in long-standing human vascular disease [37].

The most available model for study of endothelial dysfunction is hypercholesterolemia. In clinical studies hypercholesterolemic subjects demonstrate a decreased response to endothelium-dependent dilators in the coronary and peripheral vascular beds [36, 366, 367, 378-381]. Furthermore, an inverse relationship between cholesterol levels and endothelium-dependent vasodilation has also been reported in asymptomatic individuals with cholesterol levels in the high normal range [379, 382]. Probably the best demonstration of the effects of cholesterol on the brachial artery was by Tamai et al. who studied hypercholesterolemics before and following plasma LDL apheresis [383]. They found a marked increase in blood flow response to ACH following treatment whereby, LDL cholesterol levels were lowered to 30-40 mg/dL.

A good demonstration of the development of disease and its effects on artery function was performed by Zeiher and colleagues in the coronary vasculature [355]. They found hyperlipidemic patients, without atherosclerotic lesions, had already lost the endothelial dependent response to ACH, although their dilator response to increased blood flow was preserved. By the time mild atherosclerotic lesions ($\leq 30\%$ narrowing) appeared, response to ACH and flow were significantly diminished or abolished. This study raises two issues, are there separate mechanisms, with different time courses of inhibition between physical and

pharmacological stimuli, and does the extent of the neointimal lesion relate to the degree of inhibition of endothelial-dependent relaxation? Currently, the answer to the first question remains to be elucidated, but it would appear that artery vasomotion is related to the stage of atherogenesis [355, 384]. Again, it is not clear if this is related to a decreased ability for of relaxing factors to be released [385], or travel to the smooth muscle [37], or a reduction of sensitivity of the smooth muscle itself [385-387]. It is probably a combination of all of these mechanisms. Additionally, production of [388], and/or smooth muscle sensitivity to [389] vasoconstrictors may be increased.

A common factor between the pathogenesis of hypercholesterolemia and other risk factors is the production of oxygen free radicals. Endothelial cells are particularly vulnerable to oxidative stress due to their low antioxidant activity, high feritin iron, polyunsaturated fatty acid content, and exposure to the high oxygen tension of arterial blood [189]. This mechanism helps to explain the effectiveness of antioxidant treatment on restoring endothelial function in many risk factor models [74, 390, 391].

In hypercholesterolemia, the atherosclerotic process is believed to be initiated by oxygen-derived free radical-induced oxidation of lipoproteins [392]. Oxidized LDL acts via several different pathways to induce dysfunction. NO availability is reduced by oxidative stress [393], because it acts as a free radical scavenger and reacts with superoxide anions (O_2^-) to produce less active nitrate metabolites. This is advantageous in a healthy environment, as it protects the endothelial cells from the cytotoxic effects of O_2^- . In disease conditions however the balance between NO and O_2^- may be altered [394].

Oxidized LDL has also been shown to increase the expression of endothelin [395], stimulate endothelial cell monocyte adhesion [396], activate cell protein kinases, and inhibit G-

protein functions [31]. Additionally, oxidized LDL is cytotoxic to a variety of vascular cells including endothelium, smooth muscle, and fibroblasts. It increases the presence of cytokines, such as tumor necrosis factor, vascular cell adhesion molecule (VCAM1) expression, and upregulation of monocyte-directed athero-endothelial leukocyte adhesion molecule (athero-ELDM) [397], and monocyte chemoattractant protein-1 (MCP-1) which promotes monocyte migration into the sub-endothelial space in response [398]. The overall effects of these mechanisms are stimulation of foam cell formation and a pro-coagulant environment [189].

Clinical studies linking other major risk factors linked with vascular dysfunction are numerous (Figure A.12). The major mechanisms of pathogenesis in these all involve damage to, or inhibition of, the endothelium via either increased oxidative stress, decreased vasodilator synthesis, increased vasoconstrictor synthesis, increased cell adhesion molecules, and eventually smooth muscle abnormalities.

Although the associations between individual risk factors for cardiovascular disease and abnormal endothelium-dependent dilation are relatively well documented. In 1994, Celermajer and colleagues, attempted to investigate the relative importance of smoking, family history of disease, blood pressure, total cholesterol, gender and age, on flow-mediated dilation of the brachial and femoral arteries [379]. Univariate analysis confirmed the findings for individual risk factors and for the composite risk score ($r=-0.30$, $p<0.0001$). Stepwise multiple regression analysis of all variables, including the risk score, revealed an interaction effect with 40% of the variability of flow-mediated dilation accounted for by the risk factors measured.

In the absence of longitudinal or serial studies linking endothelial dysfunction with disease outcome measures, Allen et al. attempted to further determine the predictive validity of flow-mediated dilation in individuals without overt signs of CHD [403].

Figure A.12. Cardiovascular Risk Factors Linked with Vascular Dysfunction
[7, 70, 184, 221, 272, 335, 338, 348, 349, 366-369]

They extended Celermajers vasoreactivity-risk factor findings by linking flow-mediated dilation to an established epidemiological coronary heart disease (CHD) risk factor composite score algorithm. This model integrated the categorical approaches recommended by the JNC-V and NCEP programs and was developed and validated from the Framingham study population [404]. They found the univariate analyses on the association between individual CHD risk factors and FMD confirmed previous reports. In addition there was a significant relation between flow-mediated dilation and the composite risk score ($r=-0.461$, $p=0.002$) and for the 10 year CHD predicted risk ($r=-0.500$, $p=0.001$).

Taken together these findings indicate loss of vasoreactivity in the systemic arteries occurs in the pre-clinical phase of vascular disease and is associated with the interaction of risk factors known to promote the development of atherosclerosis.

A.6.5.1. Local vs. Global Dysfunction

Research evidence shows that risk factors for cardiovascular disease are associated with loss of coronary artery [380] and peripheral artery [379, 403] endothelium-dependent vasodilation in non-atherosclerotic vessels. Additionally, clinically overt atherosclerosis is associated with paradoxical vasoconstriction in the coronary beds [36] and vascular dysfunction in the brachial and femoral arteries [389, 405]. Therefore, it is logical that endothelial dysfunction maybe a generalized process across the entire vasculature. In this case non-invasive assessment of brachial artery endothelial function could serve as a useful surrogate measure for the coronary arteries. In 1995, Anderson and colleagues tested this hypothesis [406]. They assigned subjects into two groups on the basis of their coronary epicardial vasomotor response to ACH. In the normal group the coronary arteries dilated by $9\pm 11\%$ and the brachial artery by $10.8\pm 7.6\%$. In the dysfunctional group coronary constriction of $17\pm 14\%$ occurred and brachial

dilation was significantly reduced $4.8 \pm 5.5\%$ ($p < 0.01$). Additionally, in a multivariate model, the presence of coronary endothelial dysfunction was the strongest predictor of an abnormal brachial artery vasodilator response after accounting for baseline brachial artery diameters. There were no differences in response to nitroglycerin administration between the groups, indicating the differences do not appear to be related to smooth muscle structural alterations. Takase et al. modified this study protocol in a small ($n=15$) heterogeneous population of those with mild to moderate coronary artery disease and normal subjects [407]. They used serial infusions of ATP to increase flow in the coronary arteries to match the hyperemic responses in the brachial tests. Their results showed a stronger relationship between flow-mediated dilation in coronary and brachial arteries ($r = 0.78$, $p < 0.001$) when the same stimulus was used.

A.6.5.2. Treatment Strategies

Due to the complex nature of endothelial dysfunction and the existence of several different pathways for pathogenesis, it is difficult to pinpoint exactly what strategy provides the most effective treatment for a particular disease-state and stage of disease development. This section will focus on studies performed, which provide efficacy and therefore an insight into possible therapeutic mechanisms.

Two approaches to disease management include the indirect approach, by reducing the risk factors implicated in the initiation and progression of the disease process, and the direct approach, by modifying the endothelium itself. The attenuation of nitric oxide production is a key step in many of the endothelial disablement processes. Accordingly, many of the treatment processes are aimed at redressing the delicate balance of constriction/dilation, hemodynamic homeostasis, and therefore reestablishing the endothelial barrier to protect the vessel intima and further prevent long term vascular remodeling. The pathogenic processes have been outlined

previously in this section and figure A.13 illustrates the current literature involving various methods of approach. It repeats the established risk factor model from figure A.12 and provides additional information pertaining to the method of intervention resulting in an increase in endothelial-mediated dilation. Additionally, the authors of these studies are presented to provide some idea of the respective weight of papers in this area.

The efficacy of these treatments may depend upon the initial degree of perturbation, for example, Kugiyama and colleagues, treated subjects with high and low levels of remnant lipoprotein with the antioxidant alpha-tocopherol for a 4-week period. Subjects with high remnant lipoprotein levels significantly improved brachial artery flow mediated dilation from $4.1 \pm 0.3\%$ at baseline to $7.5 \pm 0.4\%$ following alpha-tocopherol versus $4.2 \pm 0.4\%$ following placebo. The treatment was ineffective in the low remnant group but this group had significantly higher dilations ($6.0 \pm 0.5\%$) original [410]. Additionally, the literature in this area is further complicated by often-conflicting reports of vascular disablement. For example, several studies report impaired vasoreactivity in patients with Type I diabetes mellitus [371, 372, 411, 414, 415], while others do not [416-418]. These discrepancies appear to be linked to differences in the selection of patients with or without the presence of other risk factors known to influence vascular reactivity and to differences in vascular assessment [416]. Another issue to consider in assessment of the efficacy of interventions may be the duration of the disease process. If endothelial damage is long-term vascular structural modifications could have occurred with associated alterations in the vessel smooth muscle and maybe therefore less responsive to treatment.

In order to expand upon the role of exercise training in endothelial function from section 5 an examination of the clinical literature will be presented. In Section 5, exercise training was

Figure A.13. The Indirect Method of Treatment
[272, 273, 375-380]

linked with direct adaptations to endothelial function such as, reduced sensitivity to NE [232, 260] and attenuated NE release [261], and an increase in eNOS [53], mRNA [266, 276-278], and associated endothelial derived relaxing factor proteins [266, 274, 276]. Vascular remodeling has also been shown following exercise training [38]. These changes contribute to increased arterial vasodilatory response to various stimuli [53, 232, 233, 260, 262-268, 270-272].

Indirect mechanisms by which exercise training can reduce endothelial dysfunction are shown in figure A.14. This illustrates the potential influence of exercise training on established risk factors. Central cardiovascular changes to training are also outlined in Section 5.

Due to the relatively recent discovery of the roles of the endothelium there is a dearth of longitudinal studies examining the effects of exercise training on vascular function. Several studies were outlined in Section 5 [280, 281, 284, 285, 373, 420]. Of particular importance here are studies involving changes in endothelial function in clinical models.

For the purpose of this review aging will be considered as a disease state. Additionally, endothelial function traditionally decreases with age [369, 379, 421, 422]. However this could in part, be related to changes in lipoprotein profile, insulin sensitivity, and blood pressure. Several studies have been performed during the last 2 years demonstrating enhanced or preserved endothelial vasoreactivity in healthy endurance-trained older men [412, 413, 423, 424].

Cross-sectional studies unanimously demonstrate significantly greater endothelium-dependent dilation in elderly physically trained subjects compared to healthy, sedentary, age-matched controls [412, 413, 423, 424]. The average age of subjects combined across studies was approximately 76yr. (n=29). Interestingly, in studies where multivariate regression analyses were performed VO_{2peak} was a significant predictor for dilation whereas lipids were not [412, 413].

Figure A.14. Possible Indirect Mechanisms by which Exercise Training May Improve Endothelial Function
[178, 386]

There were also no differences between training groups for endothelium-independent dilator responsiveness [412, 413, 423, 424].

The current literature shows only two longitudinal studies involving flow-mediated improvement in vascular function training in the elderly. In 1990 Martin et al. trained 9 men and 10 women aged 64 ± 3 yr. by walking/jogging at 70-90% $\text{VO}_{2\text{peak}}$ for 45 minutes on 3-5 days per week for approximately 6 months. They used plethysmography to examine calf blood flow pre- and post-training. Vasodilatory capacity to occlusion combined with heel-raise exercise, increased by 25% in males and by 21% in females following training [425]. In 2000, DeSouza and colleagues, walk-trained 13, previously sedentary, middle aged and older healthy men for 3 months. Following training ACH-mediated vasodilation increased by 30% versus baseline. In fact, when the authors compared these results to previous cross-sectional results between young and older subjects they found no difference in endothelium-mediated dilation between the post-training group and the young subjects. They concluded regular aerobic exercise can prevent the age associated loss in endothelium-dependent vasodilation and restore levels in previously sedentary middle aged and older healthy males [423].

Heart failure is another population in which increases in endothelial function following exercise training have been documented [286, 287]. Hornig et al, performed a cross-sectional study demonstrating reduced flow-mediated dilation in the forearm radial artery of 12 congestive heart failure subjects (NYHA class III) compared with 7 age-matched controls (41 ± 8 yr.) ($8.6 \pm 0.9\%$ vs. $13.5 \pm 0.7\%$ respectively $p < 0.05$) [286]. The heart failure subjects' non-dominant arms were then trained for 4 week using handgrip exercise at a rate of 30 contractions per minute for 30 minutes daily for 4 weeks at an intensity of 70% of a previously tested 3 minute maximum. Following training, flow-mediated vasoactivity increased by 58% ($13.6 \pm 0.9\%$)

compared to baseline. These values were similar to the untrained healthy population. Interestingly, this improvement could be abolished by L-NMMA administration. Finally, a 6-week no-training period was designed following the training. Vasoactivity in the heart failure subjects returned to near pre-training values ($8.6 \pm 0.9\%$). No differences were observed with administration of NTG or for the untrained dominant arms across the study time-period. These findings were supported Katz et al, who performed an identical training program for 8 weeks in 12 similar heart failure subjects [287]. Blood flows increased from baseline to ACH infusions only in the trained limb (8.6 ± 1.3 to 16.6 ± 2.0 ml/min/100ml/tissue), assessed by strain-gauge plethysmography.

Similar results have been shown in several other heart failure studies [426-428] and in one study involving walk training in mildly hypertensive subjects [429]. It is therefore reasonable to suggest exercise training can improve endothelial-dependent vasoactivity in these populations. Given previous studies this process is most likely mediated by a shear-induced up regulation of the nitric oxide synthase pathway and is specific to the tissues undergoing training. Additionally, this process is reversible when training ceases.

A.7. References

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APPENDIX B. CONSENT FORM

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study:

The time course of vascular adaptations following localized short-term exercise training

What you should know about a research study

- We give you this consent form so that you may read about the purpose, risks and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary.
- By signing this consent form, you agree to participate in the study as it is described.

1- Who is doing the study?

Investigator Information:

Principal Investigator: Michael A. Welsch, Ph.D.
Phone No: 225/578-9143

Medical Investigator: Frank Greenway, M.D.
Day Phone: 225/763-2576
24-hr. Emergency Phone Nos.:
225/763-2576 (Weekdays 8:00a.m.-5:00 p.m.)
225/765-4644 (After 5:00 p.m. and Weekends)

Co-Investigators: Jason Allen, M.Ed.

Dr. Michael Welsch directs this study, which is under the medical supervision of Dr. Frank Greenway. We expect about 15 people will be in this study. The study will take place over a period of 12 weeks. Your expected time in this study will be 6 weeks. *This study is developed at the PBRC and is not part of a national study.*

2- Where is the study being conducted

This study is being conducted at the Pennington Biomedical Research Center Outpatient Clinic, 6400 Perkins Road, Baton Rouge, LA 70808 and the Department of Kinesiology at Louisiana State University, 112 Huey P. Long Field House, Baton Rouge, LA 70803.

3- What is the purpose of this study?

You have been invited to participate in a research study to investigate how quickly your forearm blood vessel changes during a 4-week exercise program.

4- Who is eligible to participate in the study? Who is ineligible?

You are eligible to participate in this study if you are:

- Female (not nursing or pregnant), or male, 18 years of age or older
- Apparently healthy

You are ineligible to participate in this study if you have any of the following conditions:

- Current smoker
- Kidney disease
- Liver disease
- Gout
- Anemia
- High cholesterol
- High blood pressure
- Diabetes
- Acute medical/congestive conditions
- Active infection
- Poor circulation or previous arm surgery
- Currently on medications with known blood vessel effects (e.g. anti-inflammatory or cardiovascular medications)

5- What will happen to you if you take part in the study?

If you are selected to participate in this study it will involve 25 visits (11 visits to the Pennington Biomedical Research Center Clinic (PBRC) and 14 additional visits to the Department of Kinesiology at Louisiana State University (LSU)), over a period of 6-weeks. The total number of tests performed over the entire study includes...

- 10 forearm dilation ultrasound scans on each arm
- 6 MVC (handgrip strength tests) and forearm circumference assessments
- 3 venous blood draws (approx. 2 tablespoons of blood)
- 2 $\text{VO}_{2\text{peak}}$ arm ergometry protocols (handcrank tests)
- 2 anthropometric assessments (height, weight)
- 20 forearm resistance-training sessions (20 min handgrip training)

During the first week of the study you will be required to attend 3 visits at PBRC and 1 visit at LSU.

- On your first visit you will attend the PBRC clinic, where you will be interviewed and undergo a standard PBRC clinic screening this includes height, weight, blood pressure and body fat measures. Additionally, you will be given health and activity questionnaires to complete. Finally, you will be asked to provide 2 tablespoons of blood for the determination of variables known to affect your blood vessels. The total time for this visit will be approximately 60 minutes.
- On your second visit you will again attend the PBRC clinic and will be examined by a physician. The total time for this visit is approximately 30 minutes.
- On your third visit to PBRC you will be escorted to a quiet room and asked to lie down on an examination bed. Subsequently, a laboratory technician will place 4 electrodes and 2 blood pressure cuffs on your chest and both arms, respectively. The electrodes are used to examine your heartbeat. The cuffs are used to measure blood pressure in one arm and change blood flow to the hand in the other arm to allow us to test how your blood vessels react. After all the equipment is in place you will be given a 15-minute rest period. During this time an ultrasound technician will be taking pictures of a blood vessel (Brachial artery) in your arm. After a clear picture has been obtained the width of your blood vessel will be measured before and after 5-minutes of forearm occlusion. Forearm occlusion will be established with the blood pressure cuff on the arm being scanned. Throughout the procedure, heart rate, and blood pressure will also be recorded. Following this, we will swap the cuffs and repeat the procedure on your other arm. This procedure is called the forearm dilation measure. Finally, you will be asked to provide 2 tablespoons of blood for the determination of variables known to affect your blood vessels. The total time for this visit will be approximately 60 minutes.
- On your fourth visit you will attend LSU. This time you will be escorted to a quiet room where your weight and height are measured. Additionally, the circumference of both your forearms will be measured and you will be asked to perform a handgrip strength test. This will involve squeezing a hand gripper as tightly as you can. Each hand will be tested 3 times. These tests will be done once per week for the 6 weeks of the study duration, to check if you are getting stronger and if the muscle is getting bigger. Finally, you will perform an arm ergometer test. This involves sitting and performing an action similar to pedaling a bicycle but you will be using your arms rather than your legs. The test involves you arm cranking at a set speed (50 rpm) and resistance (0.5kp) for 2 minutes. After the initial 2 minutes the resistance will be increased by 0.25kp every subsequent 2 minutes until you reach a point that you want to or have to stop. This arm ergometer test will be repeated at the end of the 6-week trial. This visit is expected to last approximately 40 minutes.

The next 4 weeks of the study (weeks 2-5) are all very similar in terms of what you will be asked to do.

- On Mondays and Fridays you will attend PBRC and Tuesday through Thursday you will attend LSU.
- On Mondays and Fridays, at PBRC, you will undergo the forearm dilation measure. During the third week of the study, on one of these days, you will also be asked to provide 2 tablespoons of blood.

- Every day, Monday through Friday, you will be asked to perform handgrip training for a period of 20 minutes. This involves gripping and releasing the handgripper at a rate of 15 times per minute at a resistance set at 60 percent of your last measured maximum (performed the previous week).
- Additionally, on Wednesdays you will perform the handgrip strength test to check if you are getting stronger.
- These visits are expected to last approximately 70 minutes on Mondays and Fridays, 20 minutes on Tuesdays and Thursdays, and 30 minutes on Wednesdays.

During the final week of the study (week 6) you will be required to make 1 visit to PBRC and 1 visit to LSU.

- The PBRC visit will involve a forearm dilation study and a blood draw (2 tablespoons). This visit will take approximately 60 minutes.
- The LSU visit will involve a handgripper strength test, measurements of forearm circumferences, and the handcrank test. This visit will take approximately 60 minutes.

6- What are the possible risks and discomforts?

The possible risks and discomforts of participation in this study include...

- **Inflation of the blood pressure cuff on the forearm may cause some temporary discomfort in the forearm and hand. If the discomfort is too severe you may terminate the procedure immediately by asking the technician to stop the test. Temporary numbness and tingling in the hand similar to the sensation of having your hand “fall asleep” may occur. There is no known risk to the use of ultrasound to measure the size of the brachial artery blood vessel. There is also no known risk to blocking blood flow in the artery through inflation of the blood pressure cuff for the 5-minute period of time. Not a single permanent adverse event has been associated with this procedure in over 30,000 examinations reported in the medical literature.**
- **The discomforts and risks of a blood test are the pain of a needle going into an arm vein, possible bruising and the remote risk of infection.**
- **The risk of cardiovascular complications during arm ergometer exercise protocols is lower than treadmill exercise tests and is estimated as less than one per 100,000 tests.**

In addition to the risks listed above, the procedures may have unknown, unforeseen or unanticipated side effects. There is always the possibility that you will have a reaction that is currently not known and not expected.

7- What are the possible benefits?

We cannot promise any benefits from your being in the study. However, possible benefits include helping the investigators in gathering information leading to better treatments for cardiovascular disease. All clinic visits, physical examinations, laboratory

tests, electrocardiograms, and any other procedures associated with the conduct of this study are free if you participate. Information received from these tests, such as information about your blood pressure or cholesterol for example will be available to you.

8- If you do not want to take part in the study, are there other choices?

You have the choice at any time to not participate or to withdraw from this research study. A decision to not participate will not jeopardize in any way your medical treatment at your medical facility at this time or in the future.

9- If you have any questions or problems, whom can you call?

If you have any questions about your rights as a research volunteer, you should call the Institutional Review Board Office at 225/763-2693 or Dr. Claude Bouchard, Executive Director of PBRC at 225/763-2513. If you have any questions about the research study, contact Dr. Michael Welsch (PI) at 225/578-9143. If you think you have a research-related injury or medical illness, you should call Dr. Frank Greenway at 225/763-2576 (phone number) during regular working hours. After working hours and on weekends you should call the answering service at 225/765-4644. The on-call physician will respond to your call.

10- What information will be kept private?

Every effort will be made to maintain the confidentiality of your study records. However, someone from the Food and Drug Administration, the Pennington Biomedical Research Center, and *Louisiana State University Department of Kinesiology* (the sponsor) may inspect and/or copy the medical records related to the study. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11- Can your taking part in the study end early?

Dr. Greenway or the study sponsor can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include an adverse event, injury or medical condition which may place you at risk of further complications if you continue to participate, failure to keep your scheduled appointments, early termination or other administrative reasons. Your participation in this research study is voluntary. You have the right to refuse to participate or to withdraw from participation at any time for any reason. Your refusal to participate or your withdrawal from the study will involve no penalty or loss of entitled benefits, nor affect your ongoing medical care. If you decide to withdraw from the study, please notify the study director immediately.

12- What if information becomes available that might affect your decision to stay in the study?

During the course of this study there may be new findings from this or other research, which may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13- What charges will you have to pay?

None

14- What payment will you receive?

None

15- Will you be compensated for a study-related injury or medical illness?

No form of compensation for medical treatment is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should you require ongoing medical treatments, they must be provided by community physicians and hospitals.

16- Signatures

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the consent form.

Signature of Volunteer

Date

Social Security No. of Volunteer

Signature of Person Administering Informed Consent

Date

Investigator (Dr. Michael Welsch)

Date

Medical Investigator (*Dr. Frank Greenway*)

Date

APPENDIX C. DATA SPREADSHEETS

Subject	Visit	Arm	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs chg pk
1	1	1	3.896827	3.854639	3.973369	3.970523	63.83668	52.37234	103.1036	85.1739	77.67443	1.964216	1.891171	1.964216	96.86658	0.076542	0.073696	0.076542
1	3	1	3.776527	3.814683	4.146876	4.091078	91.38093	63.62305	125.3524	110.691	101.3866	9.806616	8.329098	9.806616	97.0236	0.37035	0.314551	0.37035
1	4	1	3.871922	3.806655	4.073416	3.946046	70.00085	52.76798	126.63	111.7439	99.65974	5.203978	1.914397	5.203978	139.9751	0.201494	0.074124	0.201494
1	5	1	3.971584	4.00247	4.134223	4.085166	78.97309	54.65734	116.5522	98.11097	93.45377	4.095048	2.859869	4.095048	113.2417	0.162638	0.113582	0.162638
1	6	1	3.965458	3.832573	4.083283	4.08443	73.80319	65.52178	127.0804	109.8298	108.4511	2.971282	3.000218	3.000218	93.95131	0.117825	0.118972	0.118972
1	7	1	3.808329	3.803759	4.005309	3.875275	71.38757	51.37989	134.666	101.3823	91.21733	5.172335	1.757877	5.172335	162.0987	0.19698	0.066946	0.19698
1	8	1	4.245427	4.022643	4.49088	4.50688	80.70144	51.21438	129.4968	101.5566	90.01333	5.781572	6.158443	6.158443	152.8525	0.245452	0.261452	0.261452
1	9	1	3.521167	3.35207	3.857285	3.661576	81.22193	46.3877	139.324	109.8506	95.35481	9.545631	3.987561	9.545631	200.3469	0.336118	0.140409	0.336118
1	10	1	3.950757	3.758081	4.191875	4.089249	62.77799	51.56627	160.706	92.59454	81.04194	6.103106	3.505455	6.103106	211.6494	0.241119	0.138492	0.241119
1	1	2	3.856076	3.83547	3.829676	3.828601	69.56944	61.55172	116.5275	92.71634	93.15219	-0.68463	-0.7125	-0.68463	89.31635	-0.0264	-0.02747	-0.0264
1	3	2	3.661142	3.502653	3.828582	3.828559	76.03448	65.1783	119.1466	103.9753	96.0351	4.573443	4.572802	4.573443	82.80096	0.16744	0.167417	0.16744
1	4	2	3.858227	3.911534	4.025537	3.918526	53.10628	66.72806	130.5184	105.6922	97.5902	4.336447	1.562856	4.336447	95.59744	0.16731	0.060299	0.16731
1	5	2	3.842586	3.78005	4.044015	4.049078	92.93901	62.41864	133.7931	105.3485	99.48576	5.242037	5.373796	5.373796	114.348	0.20143	0.206493	0.206493
1	6	2	3.814399	3.744966	4.074577	4.017152	89.48342	35.17316	102.7659	89.48542	79.48351	6.820959	5.315469	6.820959	192.1714	0.260179	0.202753	0.260179
1	7	2	3.8239	3.828826	3.903525	3.87343	72.59033	45.6929	139.4915	91.56113	91.38972	2.082303	1.295265	2.082303	205.2806	0.079625	0.04953	0.079625
1	8	2	3.918723	3.87426	3.925786	3.89226	46.04761	45.52	92.07696	72.94108	71.38774	0.18022	-0.6753	0.18022	102.278	0.007062	-0.02646	0.007062
1	9	2	3.61376	3.509437	3.726989	3.605215	60.87179	50.89765	126.3937	90.53368	84.34255	3.133291	-0.23645	3.133291	148.3291	0.11323	-0.00854	0.11323
1	10	2	3.824053	3.802194	4.011448	3.856563	64.49184	59.66166	145.5406	96.9026	89.48592	4.900432	0.850157	4.900432	143.9433	0.187395	0.03251	0.187395
2	1	1	3.50829	3.445598	3.757224	3.725302	88.96699	66.29516	139.0587	114.3167	108.707	7.0956	6.185709	7.0956	109.757	0.248934	0.217013	0.248934
2	3	1	3.500777	3.360025	3.858462	3.901589	123.9703	89.83918	156.7312	136.8987	128.1155	10.98855	11.44924	11.44924	74.45753	0.384685	0.400812	0.400812
2	4	1	3.851703	3.37484	3.842809	3.864577	123.7933	57.07564	155.0055	136.0354	122.414	-0.23091	0.334237	0.334237	171.5791	-0.00889	0.012874	0.012874
2	5	1	3.598527	3.253209	3.698831	3.644086	107.2425	78.2774	169.8479	148.4503	138.4491	3.0451	1.51997	3.0451	116.982	0.109305	0.05456	0.109305
2	6	1	3.548176	3.43399	3.861725	3.937216	98.96612	61.72664	141.5528	121.0456	103.4489	8.83692	10.96452	10.96452	129.322	0.313549	0.389041	0.389041
2	7	1	3.574281	3.511843	3.701765	3.57514	75.35465	60.36507	153.1225	123.806	128.122	3.566705	0.024036	3.566705	153.6607	0.127484	0.000859	0.127484
2	8	1	3.744116	3.644399	3.962427	3.845155	112.9418	58.28866	157.9408	146.3809	153.4563	-2.39023	6.105663	170.9631	0.228011	-0.08926	0.228011	
2	9	1	3.549366	3.59477	3.906676	3.803853	101.7292	66.90011	162.0814	115.1775	132.9351	10.06688	1.535122	10.06688	142.2737	0.35731	0.054487	0.35731
2	10	1	3.383267	3.537368	3.56515	3.463268	111.3971	74.49003	170.352	130.3566	139.8388	5.375941	2.364604	5.375941	128.6909	0.181882	0.080001	0.181882
2	1	2	3.192426	3.245511	3.235104	3.112955	93.10622	85.00176	121.5548	130.692	131.5522	1.336849	-2.48936	1.336849	43.00273	0.042678	-0.07947	0.042678
2	3	2	2.941282	2.744868	3.088905	3.074582	132.246	75.00079	178.1112	150.5203	140.8642	5.019015	4.532051	5.019015	137.4791	0.147623	0.1333	0.147623
2	4	2	3.301651	2.942831	3.415825	3.404445	141.3804	85.51794	178.4486	141.8982	128.6212	3.458077	3.113408	3.458077	108.668	0.114174	0.102794	0.114174
2	5	2	3.097847	3.008084	3.333976	3.278821	130.3505	97.93103	173.1089	142.7588	147.5913	7.62236	5.824505	7.62236	76.76618	0.236129	0.180434	0.236129
2	6	2	3.200964	2.996159	3.339572	3.326707	97.43738	73.96709	185.1737	145.8801	148.1099	4.330173	3.928279	4.330173	150.3461	0.138607	0.125743	0.138607
2	7	2	2.985588	2.934577	3.093306	3.042368	77.77153	57.76284	131.7437	113.293	107.2469	3.607945	1.901823	3.607945	128.0769	0.107178	0.056781	0.107178
2	8	2	3.098572	3.059383	3.289293	3.21587	97.59866	61.03693	115.1809	117.5962	111.2123	3.785538	6.155102	3.785538	88.70691	0.19072	0.117298	0.19072
2	9	2	2.989115	2.920653	3.07484	3.01645	87.77011	68.97112	170.3509	123.1066	123.803	2.867907	0.914512	2.867907	146.9887	0.085725	0.027336	0.085725
2	10	2	2.83954	2.838262	3.007106	2.953206	78.45782	68.62611	134.494	104.8359	109.8346	5.901155	4.002949	5.901155	95.98078	0.167566	0.113665	0.167566
Subject	Visit	Arm	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs chg pk
3	1	1	3.535771	3.460977	3.787529	3.729624	100.8648	94.74427	162.2468	129.1481	120.6059	7.120291	5.482599	7.120291	71.4755	0.251757	0.193852	0.251757
3	3	1	3.577063	3.608322	3.826921	3.765874	83.96552	73.62392	145.6957	131.9079	138.1116	6.985003	5.278381	6.985003	97.89181	0.249858	0.188811	0.249858
3	4	1	3.627345	3.551562	3.737255	3.626881	86.89689	77.24176	150.8797	131.2145	129.8297	3.030035	-0.01278	3.030035	95.33436	-0.10991	-0.00046	0.10991
3	5	1	3.574459	3.579804	3.65764	3.639789	96.7318	71.38616	173.4702	141.7258	135.1787	2.327087	1.827691	2.327087	143.0026	0.083181	0.06533	0.083181
3	6	1	3.823356	3.511362	4.047114	3.904683	85.17943	57.41483	144.3278	113.4587	106.7355	5.852396	2.127101	5.852396	151.3772	0.223758	0.081327	0.223758
3	7	1	3.626511	3.534809	3.656324	3.587947	83.63207	63.45202	139.1467	114.662	105.8649	0.82208	-1.06339	0.82208	119.2943	0.029813	-0.03856	0.029813
3	8	1	3.563353	3.429211	3.802014	3.661834	112.419	77.07554	176.0401	155.0162	150.5293	6.697658	2.763725	6.697658	128.3994	0.238661	0.098481	0.238661
3	9	1	3.627607	3.507269	3.81335	3.601312	83.80018	64.14021	154.8404	117.9415	108.2773	5.12027	-0.72485	5.12027	141.4093	0.185743	-0.02629	0.185743
3	10	1	3.609609	3.585061	3.800349	3.797193	94.83658	67.93694	146.4	130.706	125.8765	5.284243	5.19681	5.284243	115.494	0.190741	0.187585	0.190741
3	1	2	3.681131	3.598072	3.911148	3.868847	104.1421	107.1672	177.6786	142.2428	135.0912	6.248528	5.099421	6.248528	66.98802	0.230017	0.187716	0.230017
3	3	2	3.732563	3.594974	3.986896	3.904095	88.62136	88.10772	153.4493	133.4611	135.0035	6.813888	4.595541	6.813888	74.16096	0.254333	0.171531	0.254333
3	4	2	3.645962	3.524997	3.681373	3.607045	95.86328	77.07041	155.5248	118.1047	113.7976	0.97123	-1.06741	0.97123	101.7957	0.035411	-0.03892	0.035411
3	5	2	3.643301	3.492343	3.713357	3.608055	96.20937	108.4483	175.3697	140.6948	132.9347	1.922868	-0.96744	1.922868	61.7081	0.070056	-0.03525	0.070056
3	6	2	3.91844	3.673479	3.96594	3.933994	93.28001	82.58804	153.6277	130.6936	123.4531	1.212225	0.396945	1.212225	86.01691	0.0475	0.015554	0.0475
3	7	2	3.618795	3.488536	3.607234	3.569016	79.82909	84.82933	147.0761	120.0062	127.0751	-0.31946	-1.37557	-0.31946	73.37879	-0.01156	-0.04978	-0.01156
3	8	2	3.722325	3.70765	3.758303	3.714383	88.28664	87.94489	165.1782	117.427	114.8379	0.966546	-0.21336	0.966546	87.82009	0.035978	-0.00794	0.035978
3	9	2	3.670674	3.598855	3.734233	3.681956	93.10881	79.48465	170.3452	128.9858	120.8684	1.731534	0.307357	1.731534	114.3121	0.063559		

Subject	Visit	Arm	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs ch pk
5	1	1	4.20731	4.069474	4.308717	4.211103	92.50277	69.14196	154.9224	134.4044	114.9174	2.410257	0.090175	2.410257	130.993	0.101407	0.003794	0.101407
5	3	1	4.322536	4.287863	4.580487	4.469033	71.72961	77.41527	145.6979	112.5951	105.867	5.967569	3.389151	5.967569	88.203	0.25795	0.146497	0.25795
5	4	1	4.293341	4.260544	4.36703	4.34335	74.48319	46.21204	126.7359	114.6639	91.56145	1.71635	1.164816	1.71635	174.2486	0.073689	0.050009	0.073689
5	5	1	4.527506	4.415838	4.586912	4.668966	133.985	106.554	214.1485	168.9769	155.1741	1.312112	3.124455	3.124455	100.9765	0.059406	0.14146	0.059406
5	6	1	4.238683	4.246194	4.674033	4.50459	87.24751	97.4144	179.6569	133.9719	131.3913	10.27089	6.273346	10.27089	84.42537	0.43535	0.265907	0.43535
5	7	1	4.111188	4.131065	4.347292	4.146197	94.83903	74.15476	174.1572	148.6342	146.3885	5.742962	0.851554	5.742962	134.8565	0.236104	0.035009	0.236104
5	8	1	4.296872	4.173358	4.41341	4.243926	72.41911	82.07549	150.7126	118.8017	110.3459	2.712156	-1.23219	2.712156	83.62679	0.116538	-0.05295	0.116538
5	9	1	4.325588	4.322964	4.465412	4.412132	86.5562	77.759	129.8468	117.0855	104.6625	3.232492	2.000745	3.232492	66.98624	0.139824	0.086544	0.139824
5	10	1	4.325588	4.322964	4.465412	4.412132	86.5562	77.759	129.8468	117.0855	104.6625	3.232492	2.000745	3.232492	66.98624	0.139824	0.086544	0.139824
5	1	2	4.134644	4.236783	4.44807	4.32218	90.69793	74.57336	168.1168	136.8991	130.8642	7.580491	4.535712	7.580491	127.2186	0.313426	0.187536	0.313426
5	3	2	4.249483	4.204651	4.420872	4.441478		61.73281	141.5629	123.6216	118.1135	4.033157	4.518079	4.518079	129.3155	0.171388	0.191995	0.171388
5	4	2	4.19498	4.230068	4.330485	4.304823	80.70144	54.83192	134.8294	114.1426	110.5239	3.230153	2.618423	3.230153	145.8957	0.135504	0.109842	0.135504
5	5	2	4.546194	4.499655	4.665738	4.597313	96.72567	76.56164	152.7642	124.4875	122.4157	2.629538	1.124437	2.629538	99.53098	0.119544	0.051119	0.119544
5	6	2	4.288957	4.257687	4.393124	4.325177	85.17959	62.7605	164.6714	132.7631	134.3192	2.428729	0.844508	2.428729	162.3807	0.104167	0.036221	0.104167
5	7	2	4.170812	3.909838	4.28396	4.046099	89.83818	70.52843	143.1053	133.1226	130.0225	2.712853	-2.99012	2.712853	102.9044	0.113148	-0.12471	0.113148
5	8	2	4.242529	4.159226	4.363665	4.198016	73.11147	71.39118	146.2279	127.2509	115.5214	2.855267	-1.04922	2.855267	104.8262	0.121136	-0.04451	0.121136
5	9	2	4.468926	4.138349	4.362304	4.207931	76.38243	60.52947	207.2427	137.7659	125.1821	-2.38586	-5.84022	-2.38586	142.8321	-0.10662	-0.261	-0.10662
5	10	2												-2.38586		-0.10662	-0.261	-0.10662
6	1	1	3.911647	3.873014	3.969286	3.897997	86.21165	51.64178	128.8063	111.7267	106.3829	1.473539	-0.34896	1.473539	154.1517	0.05764	-0.01365	0.05764
6	3	1	3.882448	3.866942	4.117833	4.089259	92.24458	71.03999	133.6318	107.9553	112.4234	6.0628	5.326813	6.0628	88.10789	0.235385	0.206811	0.235385
6	4	1	3.999859	3.953525	4.228885	4.246274	100.3498	50.00295	146.9115	144.8442	142.2485	5.725871	6.160597	6.160597	193.8057	0.229027	0.246415	0.229027
6	5	1	3.947975	4.014937	4.378111	4.338627	99.66284	90.51858	148.1206	150.5198	143.2793	10.89511	9.895004	10.89511	63.63553	0.430136	0.390652	0.430136
6	6	1	3.895528	3.925906	4.273863	4.187875	110.3486	94.82946	159.8405	148.9685	144.5733	9.695891	7.472048	9.695891	68.34178	0.378335	0.292347	0.378335
6	7	1	3.843082	3.836875	4.189616	4.037122	121.0345	99.14033	171.5604	147.4172	145.8673	8.496667	5.049091	8.496667	73.04803	0.326534	0.194041	0.326534
6	8	1	3.977567	3.981505	4.209278	4.212919	116.3839	70.17326	184.8384	159.3111	154.4878	5.825444	5.916998	5.825444	163.4029	0.231711	0.235353	0.235353
6	9	1	3.977567	3.981505	4.209278	4.212919	116.3839	70.17326	184.8384	159.3111	154.4878	5.825444	5.916998	5.916998	163.4029	0.231711	0.235353	0.235353
6	10	1	3.876567	3.88888	3.972033	3.941047	91.03779	69.14137	128.9715	113.2834	117.2424	2.462645	1.341452	2.462645	86.53308	0.095466	0.06448	0.095466
6	1	2	3.899867	3.940931	4.006512	4.010473	70.26365	66.98664	132.9413	113.3804	103.8836	2.998704	3.100534	2.998704	108.6964	0.116646	0.120607	0.116646
6	3	2	4.046457	4.02074	4.193139	4.157922	75.52232	77.59578	138.4665	121.2178	112.5929	3.624956	2.754634	3.624956	78.44591	0.146682	0.111465	0.146682
6	4	2	4.237899				87.76194							1.642344				
6	5	2	4.267207	4.225258	4.2853	4.289625	88.9672	68.96595	168.9662	133.452	129.3106	0.424	0.530054	0.530054	144.9995	0.018093	0.022619	0.022619
6	6	2	4.235565	4.155232	4.293444	4.253759	102.6749	84.1389	167.3401	139.3209	133.0179	1.373615	0.428814	1.426642	105.9308	0.05788	0.018195	0.05788
6	7	2	4.203922	4.085205	4.301589	4.217693	116.3826	99.31186	165.7139	145.1898	136.7252	2.32323	0.327573	2.32323	66.86218	0.097667	0.013771	0.097667
6	8	2	4.315466	4.126976	4.378998	4.316897	103.2876	69.82972	163.6236	118.2855	112.9352	1.472188	0.033166	1.472188	134.3179	0.063532	0.001431	0.063532
6	9	2	4.11403	3.942728	4.136973	4.142944	90.69706	64.48634	146.7294	109.6615	102.5024	0.510582	0.373289	1.4568	126.9229	0.022943	0.028914	0.028914
6	10	2	3.912595	3.758481	3.894948	3.968992	78.10652	59.14295	129.8353	101.0375	92.06961	-0.45102	1.441412	1.441412	119.5279	-0.01765	0.056397	0.056397
Subject	Visit	Arm	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs ch pk
7	1	1	3.069464	2.957245	3.326579	3.370671	104.6575	56.29956	162.8503	139.1511	137.2449	8.376547	9.813041	9.813041	189.6983	0.257115	0.301208	0.301208
7	3	1	3.111537	3.021293	3.659464	3.596864	124.1476	94.82789	159.4942	131.2128	122.5872	10.50651	11.25413	10.50651	68.19328	0.347927	0.285327	0.347927
7	4	1	3.159553	3.202581	3.508307	3.545323	99.49271	90.0135	169.3191	138.4487	131.7325	11.03807	12.20962	12.20962	88.10409	0.348754	0.38577	0.38577
7	5	1	3.639685	3.51345	3.962283	3.928784	108.6232	87.2458	167.0821	155.8742	139.836	8.863355	7.942977	8.863355	91.50733	0.322598	0.280909	0.322598
7	6	1	3.13436	2.970193	3.350588	3.392999	141.7243	71.72783	174.1384	154.1471	160.3656	6.898629	8.251737	8.251737	142.7766	0.216228	0.258639	0.258639
7	7	1	3.482447	3.291375	3.686851	3.789995	128.4487	79.83132	167.2431	146.3811	133.4538	5.869534	8.831374	8.831374	109.4957	0.204403	0.307548	0.307548
7	8	1	3.297694	3.082633	3.552644	3.65903	121.2133	78.45473	153.4521	136.5546	135	7.731179	10.95724	10.95724	95.59323	0.254951	0.361336	0.361336
7	9	1	3.133731	3.046871	3.503904	3.452701	102.7601	48.27903	162.0725	131.387	102.9321	11.81252	10.1786	11.81252	235.6996	0.370173	0.31897	0.370173
7	10	1	3.076828	3.085342	3.482081	3.429398	132.933	97.93346	153.6348	141.3898	141.7293	13.17113	11.45888	13.17113	66.87669	0.405253	0.35257	0.405253
7	1	2	3.072604	2.92794	3.403001	3.318238	89.40549	71.29405	167.8549	123.373	116.1295	10.75298	7.994302	10.75298	153.9018	0.330396	0.245633	0.330396
7	3	2	3.194749	2.994939	3.518343	3.501801	122.9515	87.24313	153.4624	143.969	125.1854	10.12895	9.611143	10.12895	75.90196	0.323594	0.307052	0.323594
7	4	2	3.293038	3.200628	3.548814	3.668797	81.89692	83.10379	145.8673	122.0739	125.6927	7.767171	11.4107	11.4107	75.52421	0.255776	0.375759	0.375759
7	5	2	3.661041	3.632121	3.942047	3.870629	111.3859	97.93578	159.3195	145.3567	139.3146	7.675575	5.72482	7.675575	62.67757	0.281006	0.209588	0.281006
7	6	2	3.145393		3.35239	3.393508	131.2098	68.27799	167.2458	147.5945	153.6449	6.580974	7.888209	7.888209	144.9484	0.206998	0.248115	0.248115
7	7	2	3.384329	3.060953	3.488122	3.512108	112.7663	66.21139	169.6724	146.5568	140.3509	3.066872	3.775612	3.775612	156.2586	0.103793	0.127779	0.127779
7	8	2	3.358729	3.124503	3.510364	3.51141	102.7645	75.95087	166.3889	143.8829	136.2118	4.525781	4.551715	4.880151	123.3007	0.151635	0.152681	0.152681
7	9	2	3.333129	3.224052	3.532607	3.510712	92.7628	85.69035	163.1054	141.209	132.0726	5.984689	5.327818	5.984689	90.34281	0.199477	0.177583	0.199477
7	10	2	3.283876	3.127172	3.504567	3.478666	103.7959	47.2445	148.9681	133.2875	135.6905	6.720441	5					

Subject	Visit	Arm	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs ch pk
10	1	1	2.706295	2.692113	2.924518	2.895363	59.66449	36.38187	141.8131	91.98958	92.67705	8.063517	6.986208	8.063517	289.8967	0.218223	0.189067	0.218223
10	3	1	2.94831	2.768477	3.17769	3.047828	50.86497	38.79701	147.9379	98.10869	92.06929	7.780046	3.375418	7.780046	281.3125	0.22938	0.099518	0.22938
10	4	1	2.848709	3.157949	3.15372	72.41461			135.6952	105.8716	94.6558	10.85541	10.70698	10.85541		0.309239	0.305011	0.309239
10	5	1	2.975431	2.890033	3.306803	3.256169	78.44864	47.24138	146.3862	109.8325	106.3843	11.13695	9.435197	11.13695	209.8687	0.331372	0.280738	0.331372
10	6	1	2.907725	2.852902	3.150099	3.067344	67.24183	57.07001	144.8303	118.9735	111.2104	8.335501	5.489449	8.335501	153.7765	0.242373	0.159618	0.242373
10	7	1	3.084546	2.811311	3.395872	3.367025	77.76246	48.10583	163.4565	126.9083	121.9035	10.09312	9.157894	10.09312	239.7852	0.311327	0.282479	0.311327
10	8	1	3.064814	2.92295	3.439188	3.380243	68.625	44.13928	155.8672	113.1982	105.7823	12.22899	10.29932	12.22899	222.3749	0.374374	0.31543	0.374374
10	9	1	3.045082	3.03459	3.482504	3.393462	66.731	48.62133	148.2778	99.48814	89.66114	14.36487	11.44074	14.36487	204.9646	0.437422	0.34838	0.437422
10	10	1	2.877541	2.831573	3.27702	3.221991	62.93198	35.00087	136.208	109.139	91.041	13.88266	11.97029	13.88266	289.156	0.399479	0.34445	0.399479
10	1	2	2.725785	2.621996	2.892266	2.874354	73.02307	63.88118	159.3995	111.3843	103.3629	6.107613	5.450474	6.107613	149.3533	0.16648	0.148568	0.16648
10	3	2	3.021749	2.670737	3.024038	2.94831	65.17606	90.8637	145.1856	101.73	101.5638	0.075728	-2.43035	0.075728	59.78395	0.002288	-0.07344	0.002288
10	4	2	2.927802	2.785048	2.958632	2.850353	72.58703	88.97388	180.364	136.559	127.42	1.052997	-2.64529	1.052997	102.7157	0.03083	-0.07745	0.03083
10	5	2	2.972198	2.8232	3.142076	3.106005	85.00589	75.00994	154.3325	134.6608	120.6909	5.715587	4.501955	5.715587	105.7494	0.169879	0.133807	0.169879
10	6	2	3.019549	2.755506	3.061464	3.055076	82.93566	77.58697	149.6578	113.7957	107.9321	1.388109	1.17655	1.388109	92.89035	0.041915	0.035527	0.041915
10	7	2	2.933355	2.684607	3.163154	3.053322	78.62438	88.28123	158.4537	117.2541	113.626	7.834033	4.089768	7.834033	79.48742	-0.2298	0.119967	0.2298
10	8	2	2.782737	2.75823	3.042297	2.924247	80.1791	92.5894	170.1837	121.7285	106.035	9.327501	5.08528	9.327501	83.80472	0.25956	0.14151	0.25956
10	9	2	2.998745	2.921402	3.322557	3.288595	66.03896	72.76681	139.1422	109.663	103.7979	10.79826	9.66571	10.79826	9.21656	0.323812	0.28985	0.323812
10	10	2	2.851355	2.748407	3.126714	2.982996	64.14217	64.48747	166.5521	113.9744	103.6221	9.657137	4.616789	9.657137	158.2074	0.275359	0.131641	0.275359
12	1	1	2.81709	2.92902	3.007223	2.982072	89.1406	67.67427	140.356	107.5048	103.3631	6.749277	6.749277	6.749277	112.17	0.190133	0.164982	0.190133
12	3	1	2.836097	2.932417	2.958313	2.957754	82.93695	73.75633	147.939	119.8278	115.1751	4.309307	4.289601	4.309307	99.98339	0.122216	0.121657	0.122216
12	4	1	2.96444	2.987062	3.140477	3.217699	105.5228	95.69493	152.2634	124.6583	126.8996	5.938268	8.54323	8.54323	91.1336	0.176036	0.253259	0.176036
12	5	1	2.931274	2.919054	3.113595	3.169108	83.79381	91.03776	150.6971	113.11	105.6911	6.219878	8.113685	8.113685	65.53249	0.182322	0.237834	0.182322
12	6	1	2.777629	2.646755	3.009417	2.936034	73.45348	58.97031	133.6298	100.1739	106.7255	8.344846	5.702919	8.344846	128.6052	0.231789	0.158406	0.231789
12	7	1	2.864291	2.887275	3.192505	3.131366	93.454	57.75914	137.0852	112.5896	110.357	11.45882	9.324296	11.45882	137.3395	0.328214	0.267075	0.328214
12	8	1	2.824777	2.956099	2.973401	2.949536	82.06969	58.62069	148.1044	109.4833	107.0833	5.12375	6.200209	9.767668	152.6488	0.144924	0.12106	0.144924
12	9	1	2.855849	2.890186	3.035273	3.098439	87.93947	60.00192	144.4894	116.8998	113.2843	6.282696	8.494512	8.494512	140.8079	0.179424	0.24259	0.179424
12	10	1	3.079586	3.094036	3.346801	3.296024	78.28348	68.45389	134.8333	107.4149	103.9692	8.677003	7.028161	8.677003	96.96944	0.287216	0.216438	0.287216
12	1	2	3.366765	3.291544	3.373681	3.265295	62.41646	78.53746	110.9502	85.26184	79.65647	0.204808	-3.01387	0.204808	45.87753	0.006895	-0.10147	0.006895
12	3	2	3.3905	3.343968	3.459771	3.482314	75.34859	76.38129	154.3246	112.2466	99.31156	2.043083	2.707962	2.043083	102.045	0.069271	0.091813	0.069271
12	4	2	3.496358	3.402628	3.605809	3.468149	91.72544	65.86252	136.558	117.9442	112.7613	3.13042	-0.80681	3.13042	107.338	0.109451	-0.02821	0.109451
12	5	2	3.501323	3.425333	3.449633	3.426886	73.79511	85.0007	140.0004	95.51882	86.20759	-1.47629	-2.12596	-1.47629	64.70503	-0.05169	-0.07444	-0.05169
12	6	2	3.269614	3.258558	3.332955	3.387607	69.13879	94.3136	150.0148	108.6261	94.49057	1.937261	3.608778	3.608778	59.05952	0.063341	0.117993	0.063341
12	7	2	3.530054	3.527716	3.454641	3.446677	68.10911	62.93575	116.3798	103.1058	91.21504	-2.1363	-2.3619	-2.1363	84.91847	-0.07541	-0.08338	-0.07541
12	8	2	3.283144	3.255705	3.478335	3.34327	68.79525	53.27586	109.8303	85.17828	76.55484	5.945238	1.831355	1.831355	106.154	0.195191	0.060126	0.195191
12	9	2	3.557771	3.343933	3.487613	3.419469	70.86934	69.65731	139.6167	106.217	99.66054	-1.97196	-3.88732	-1.97196	100.4337	-0.07016	-0.1383	-0.07016
12	10	2	3.613128	3.406517	3.592931	3.524854	78.6218	68.5669	145.6974	96.90057	99.14548	-0.55898	-2.44316	-0.55898	132.793	-0.0202	-0.08827	-0.0202
Subject	Visit	Arm	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs ch pk
13	1	1	3.097054	3.026505	3.268402	3.262778	129.5773	83.27958	184.6699	144.0554	131.0408	5.532611	5.35103	5.532611	124.7875	0.171348	0.165724	0.171348
13	3	1	3.489266	3.423479	3.670604	3.592872	98.10647	75.52433	164.6813	132.7624	121.3805	5.197041	2.969288	5.197041	118.0506	0.181339	0.103606	0.181339
13	4	1	3.335179	3.232851	3.508515	3.428759	137.4159	65.51922	140.6917	127.5902	126.5536	5.197215	2.805852	5.197215	114.7335	0.173336	0.09358	0.173336
13	5	1	3.260905	3.122055	3.47626	3.418623	145.3478	90.00309	180.3515	151.3884	148.7962	6.636956	4.883988	6.636956	103.4525	0.215355	0.157719	0.215355
13	6	1	3.166631	3.01126	3.444005	3.408488	153.2797	114.487	220.0114	175.1865	171.0388	8.076697	9.62124	8.076697	92.17153	0.227375	0.221857	0.227375
13	7	1	3.311586	3.094667	3.564038	3.479545	107.9324	80.52645	192.419	144.8303	151.566	7.623313	5.071862	7.623313	138.9513	0.252453	0.167959	0.252453
13	8	1	3.257061	3.111355	3.454243	3.515649	108.7942	85.0028	179.4862	136.5545	131.2071	6.054004	7.939311	7.939311	111.1533	0.197183	0.258588	0.197183
13	9	1	3.37453	3.220792	3.537989	3.478365	107.5951	72.06979	176.8998	146.0347	141.2107	4.8439	3.077023	4.8439	145.4562	0.163459	0.103835	0.163459
13	10	1	3.187492	3.095528	3.348299	3.28113	106.2075	85.00305	194.4861	155.0025	153.7996	5.044926	2.937659	5.044926	128.799	0.160807	0.093638	0.160807
13	1	2	3.227684	3.183051	3.469416	3.443047	111.8173	74.13811	180.2638	130.35	128.7154	7.489326	6.672366	7.489326	143.5864	0.241732	0.215363	0.241732
13	3	2	3.298185	3.331967	3.4225	3.376426	86.38	79.31222	155.7113	117.2545	110.8687	3.769185	2.372241	3.769185	96.32698	0.124315	0.078241	0.124315
13	4	2	3.477418	3.521838	3.672068	3.602443	101.9051	75.34676	165.5415	136.7252	131.0483	5.597537	4.394821	5.597537	119.7062	0.19465	0.125024	0.19465
13	5	2	3.439258	3.421029	3.601356	3.548301	120.0961	82.59284	182.9474	143.8809	143.0258	4.703261	3.565531	4.703261	121.36	0.162099	0.109043	0.162099
13	6	2	3.401098	3.32022	3.530645	3.49416	138.2871	89.83893	200.3532	151.0365	155.0033	3.808985	2.73624	3.808985	123.0138	0.129547	0.093062	0.129547
13	7	2	3.303802	3.260848	3.537102	3.531142	119.6562	91.55469	171.0537	141.7252	133.6216	7.061566	6.881161	7.061566	86.83222	0.2333	0.22734	0.2333
13	8	2	3.48426	3.409317	3.663963	3.630879	102.2496	100.692	160.3624	137.7641	138.9711	5.157567	4.208034	5.157567	69.26026	0.179703	0.146619	0.179703
13	9	2	3.380558	3.43956	3.572255	3.528833	97.58932	64.48878	146.3911	120.5234	112.2414	5.670561	4.386107	5.67				

Subject	Visit	Arm	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	peak	prev/relv	abs chg 6	abs chg 9	abs ch pk
15	1	1	3.635358	3.751176	3.909914	3.810726	90.94908	59.22869	153.6315	113.3645	103.7982	7.552374	4.823962	7.552374	158.4289	0.274556	0.175368	0.274556
15	3	1	4.035264	4.018634	4.381597	4.373604	67.93834	36.72414	157.4371	115.1757	100.8644	8.582654	8.384574	8.582654	328.7021	0.346333	0.33834	0.346333
15	4	1	3.84181		4.413302	4.09257	97.76136	45.6903	193.9934	116.3865	107.2425	14.87562	6.527139	14.87562	324.5834	0.571493	0.25076	0.571493
15	5	1	3.990551	3.96464	4.382157	4.149548								9.995216		0.391605	0.158996	0.391605
15	6	1	4.139293	3.96464	4.351011	4.206526	90.34807	38.79386	155.0004	115.5224	118.7973	5.114814	1.624246	5.114814	299.5488	0.211717	0.067232	0.211717
15	7	1	3.838151	3.676389	4.046472	3.967943	80.34779	54.48489	135.8657	104.4902	92.25454	5.427629	3.381621	5.427629	149.3641	0.208321	0.129792	0.208321
15	8	1	3.927995	3.948686	4.193444	4.191848	83.79913	27.24357	151.215	114.8367	107.5906	6.757877	6.717248	6.757877	455.0484	0.265449	0.263853	0.265449
15	9	1	3.958974	4.048415	4.517087	4.176301	91.38741	61.72414	156.3903	116.8968	101.7256	14.09741	5.489492	14.09741	153.3698	0.558113	0.217328	0.558113
15	10	1	3.644906	3.439266	3.91149	3.761365	66.37976	35.86294	176.0407	114.3149	102.5891	7.31389	3.195121	7.31389	390.8709	0.266584	0.116459	0.266584
15	1	2	3.794989	3.707679	3.845827	3.783032	77.94055	83.79381	164.147	120.5175	102.0742	1.339621	-0.31505	1.339621	95.89392	0.050838	-0.01196	0.050838
15	3	2	3.665825	3.353971	3.753846	3.549544	97.58923	47.936	157.4259	106.0418	101.5618	2.401142	-3.17203	2.401142	228.4086	0.088022	-0.11628	0.088022
15	4	2	3.962651	3.680661	3.965715	3.762247	94.31346	62.42237	154.4835	101.036	113.7944	0.077324	-5.05731	0.077324	147.481	0.003064	-0.2004	0.003064
15	5	2	2.874886	2.80233	3.080855	2.988265								-0.73181		0.205969	0.113378	0.205969
15	6	2	4.121513	3.843866	4.058003	4.026973	89.65584	61.89855	174.4858	115.8646	97.59014	-1.54095	-2.29381	-1.54095	181.89	-0.06351	-0.09454	-0.06351
15	7	2	3.840727	3.753099	4.082676	4.011258	92.07221	51.89952	144.3145	95.52001	98.80317	6.299584	4.440089	6.299584	178.0651	0.24195	0.170532	0.24195
15	8	2	3.937819	3.767049	4.108304	4.049118	65.52313	35.52145	154.8317	112.2427	106.3818	4.329427	2.826418	4.329427	335.8823	0.170485	0.111299	0.170485
15	9	2	3.624283	3.379698	4.047222	3.984644	87.24206	54.31269	176.3815	120.8671	104.1451	11.66958	9.942947	11.66958	224.7519	0.422939	0.360361	0.422939
15	10	2	3.68913	3.566296	3.903993	3.787244	71.38096	50.0051	139.8315	109.8289	91.38737	5.824194	2.659544	5.824194	179.6344	0.214862	0.098114	0.214862
16	1	1	3.397871	3.404298	3.751215	3.584855	117.0765	119.4015	183.3001	143.2847	134.4865	10.39896	5.502978	10.39896	63.49166	0.353343	0.186984	0.353343
16	3	1	3.452559	3.419624	3.849518	3.632355	119.6564	101.0375	164.8512	128.4541	127.4168	11.49751	5.207591	11.49751	63.1584	0.396958	0.179795	0.396958
16	4	1	3.395123	3.560535	4.097768	4.044787	123.9778	103.9669	156.3909	131.3811	121.3906	20.69573	19.13522	20.69573	50.42369	0.702645	0.649664	0.702645
16	5	1	3.426304		4.027005	3.739914	124.489	130.5209	194.3312	158.7978	139.4896	17.53204	9.153021	20.09786	48.88998	0.600701	0.31361	0.600701
16	6	1	3.547751	3.467939	4.016295	3.631957	126.897	109.8471	175.3668	141.56	132.7622	13.20678	2.373484	13.20678	59.64629	0.468544	0.084205	0.468544
16	7	1	3.460382	3.405741	4.165597	4.014922	126.8987	89.49965	205.7467	166.3896	145.8655	20.37971	16.02543	20.37971	129.8855	0.705216	0.554541	0.705216
16	8	1	3.538087	3.310165	4.07286	4.129832	115.3474	64.65749	154.1404	146.3812	129.1473	15.11475	16.725	16.725	138.3953	0.534773	0.591745	0.591745
16	9	1	2.874886	2.80233	3.080855	2.988265								15.72969		0.205969	0.113378	0.205969
16	10	1	3.408108	3.386308	3.765655	3.729818	115.0003	111.2079	173.4487	133.1037	121.2074	10.49105	9.439537	10.49105	55.96791	0.357546	0.32171	0.357546
16	1	2	3.533973	3.320106	3.572366	3.379838	82.68011	87.07377	158.2868	121.4697	113.4528	1.086395	-4.36152	1.086395	81.73805	0.038393	-0.15413	0.038393
16	3	2	3.648982			3.503958	95.87108					108.4505		-3.97438		-3.64898	-0.14502	-0.14502
16	4	2	3.751394	3.677658	3.776772	3.53186	92.41921	92.76439	154.8311	109.6731	111.3865	0.676499	-5.85207	-3.5099	66.90785	0.025378	-0.21953	0.025378
16	5	2	3.752567	3.612544	3.731299	3.769604	116.2102	106.3804	145.8741	116.3826	114.4848	-0.56675	0.454017	0.454017	37.12497	-0.02127	0.017037	0.017037
16	6	2	3.628568	3.381354	3.909257	3.690542	100.6911	112.9336	157.0932	137.2512	151.0488	7.73552	1.707947	7.73552	39.10226	0.280689	0.061974	0.280689
16	7	2	3.736951	3.512594	3.92534	3.703065	97.41531	83.10805	150.8732	125.5196	109.8321	5.04124	-0.90677	5.04124	81.53857	0.188389	-0.03389	0.188389
16	8	2	3.756639	3.621449	4.097973	4.044779	92.42409	97.58771	174.8345	136.7294	125.6897	9.086144	7.670158	9.086144	79.15632	0.341334	0.28814	0.341334
16	9	2	2.874886	2.80233	3.080855	2.988265								10.06989		0.205969	0.113378	0.205969
16	10	2	3.778034	3.61631	4.195644	3.875926	84.14146	85.86276	173.97	118.9688	116.3875	11.05364	2.591098	11.05364	102.614	0.41761	0.097893	0.41761

Subject 01	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.984479	3.891321	4.013361	4.076812	64.22414	60.08621	103.4484	87.7587	82.41389	0.724868	2.317317	72.16655	62	64
b2	3.809175	3.817958	3.933377	3.864234	63.44922	44.65848	102.7589	82.5891	72.93497	3.260601	1.445413	130.0995	58	61
w2	3.776527	3.814683	4.146876	4.091078	91.38093	63.62305	125.3524	110.691	101.3866	9.806616	8.329098	97.0236	67	66
w3a	3.871922	3.806655	4.073416	3.946046	70.00085	52.76798	126.63	111.7439	99.65974	5.203978	1.914397	139.9751	58	63
w3b	3.971584	4.00247	4.134223	4.085166	78.97309	54.65734	116.5522	98.11097	93.45377	4.095048	2.859869	113.2417	62	60
w4a	3.965458	3.832573	4.083283	4.08443	73.80319	65.52178	127.0804	109.8298	108.4511	2.971282	3.000218	93.95131	61	60
w4b	3.808329	3.803759	4.005309	3.875275	71.38757	51.37989	134.666	101.3823	91.21733	5.172335	1.757877	162.0987	67	65
w5a	4.052609	4.022643	4.253746	4.206041	80.70144	51.21438	129.4968	101.5566	90.01333	4.963143	3.786012	152.8525	62	62
w5b	3.396324	3.35207	3.808711	3.548575	81.22193	46.3877	139.324	109.8506	95.35481	12.14214	4.48281	200.3469	66	67
end	3.814014	3.758081	3.988157	3.933183	62.77799	51.56627	160.706	92.59454	81.04194	4.565877	3.124521	211.6494	58	59
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	64	64	62	62	104	100	100	102	72	66	68	72		
b2	59	59	62	57	114	116	114	116	70	72	70	76	26	52.3
w2	68	67	65	67	105	102	102	100	70	70	70	72		53.3
w3a	60	61	58	61	105	107	107	102	75	75	75	75	26.7	49
w3b	60	61	58	58	116	114	116	114	70	70	72	72	26.25	53
w4a	60	61	58	60	104	102	102	100	70	70	70	70	25.95	53.67
w4b	64	62	63	63	114	114	114	114	74	76	76	78	26.75	52.3
w5a	64	65	64	59	110	110	110	108	70	72	74	72		
w5b	64	65	68	64	110	116	118	118	74	74	74	74		
end	61	59	60	60	106	108	108	108	70	76	76	74		
Subject 01	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.846285	3.806119	3.829043	3.824752	68.6208	64.65517	112.02	90.43144	88.27654	-0.44829	-0.55984	73.25756	62	65
b2	3.865867	3.864821	3.83031	3.832451	70.51808	58.44828	121.035	95.00124	98.02785	-0.91977	-0.86438	107.0805	58	56
w2	3.661142	3.502653	3.828582	3.828559	76.03448	65.1783	119.1466	103.9753	96.0351	4.573443	4.572802	82.80096	66	67
w3a	3.858227	3.911534	4.025537	3.918526	53.10628	66.72806	130.5184	105.6922	97.5902	4.336447	1.562856	95.59744	59	59
w3b	3.842586	3.78005	4.044015	4.049078	92.93901	62.41864	133.7931	105.3485	99.48576	5.242037	5.373796	114.348	59	61
w4a	3.814399	3.744966	4.074577	4.017152	89.48342	35.17316	102.7659	89.48542	79.48351	6.820959	5.315469	192.1714	57	58
w4b	3.8239	3.828826	3.903525	3.87343	72.59033	45.6929	139.4915	91.56113	91.38972	2.082303	1.295265	205.2806	68	60
w5a	3.918723	3.87426	3.925786	3.89226	46.04761	45.52	92.07696	72.94108	71.38774	0.18022	-0.6753	102.278	66	62
w5b	3.61376	3.509437	3.726989	3.605215	60.87179	50.89765	126.3937	90.53368	84.34255	3.133291	-0.23645	148.3291	65	65
end	3.824053	3.802194	4.011448	3.856563	64.49184	59.66166	145.5406	96.9026	89.48592	4.900432	0.850157	143.9433	58	58
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	61	64	62	61	102	98	98	96	68	70	66	70		
b2	57	57	60	58	112	112	112	110	72	72	70	70	25.6	50
w2	64	62	64	77	95	102	102	102	70	70	70	75		51.7
w3a	59	59	60	59	102	102	102	102	75	75	75	75	26.4	52.67
w3b	59	62	59	58	116	116	114	114	70	70	70	68	25.95	51
w4a	58	60	61	59	104	102	102	102	70	70	70	70	26.15	55.9
w4b	63	63	63	64	110	112	112	112	68	70	72	72	26.6	54.67
w5a	61	61	60	61	100	108	104	104	72	72	72	70		
w5b	65	64	64	64	110	110	114	116	74	74	74	76	26	53
end	57	58	57	57	108	108	108	108	74	76	76	74		

Subject 02	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.482977	3.488997	3.829327	3.783581	100.865	75.51877	154.8294	112.762	126.8968	9.944085	8.630653	105.021	55	55
b2	3.533602	3.402199	3.68512	3.667024	77.06897	57.07155	123.2881	115.8714	90.51724	4.287924	3.775794	116.0237	64	58
w2	3.500777	3.360025	3.885462	3.901589	123.9703	89.83918	156.7312	136.8987	128.1155	10.98855	11.44924	74.45753	69	64
w3a	3.851703	3.37484	3.842809	3.864577	123.7933	57.07564	155.0055	136.0354	122.414	-0.23091	0.334237	171.5791	75	72
w3b	3.589527	3.253209	3.698831	3.644086	107.2425	78.2774	169.8479	148.4503	138.4491	3.0451	1.51997	116.982	68	65
w4a	3.548176	3.43399	3.861725	3.937216	98.96612	61.72664	141.5528	121.0456	103.4489	8.83692	10.96452	129.322	58	55
w4b	3.574281	3.511843	3.701765	3.57514	75.35465	60.36507	153.1225	123.806	128.122	3.566705	0.024036	153.6607	52	55
w5a	3.734416	3.644399	3.962427	3.645155	112.9418	58.28866	157.9408	146.3809	153.4583	6.105663	-2.39023	170.9631	64	56
w5b	3.549366	3.59477	3.906676	3.603853	101.7292	66.90011	162.0814	115.1775	132.9351	10.06688	1.535122	142.2737	58	51
end	3.383267	3.537368	3.56515	3.463268	111.3971	74.49003	170.352	130.3566	139.8388	5.375941	2.364604	128.6909	52	56
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	54	55	55	57	98	100	100	98	70	62	62	68		
b2	66	60	58	58	118	124	120	118	74	78	78	78	24.55	39
w2	70	70	68	65	115	115	115	115	70	72	72	72	24.4	32.7
w3a	69	70	68	68	115	115	115	115	66	66	65	65		38.3
w3b	65	66	65	68	116	116	116	114	62	70	70	72	25.35	38.3
w4a	60	58	60	63	100	104	98	100	64	70	68	66		
w4b	57	61	56	52	108	108	104	102	70	68	68	70	24.8	42
w5a	59	57	58	60	104	100	102	100	70	70	72	70	24.4	43.3
w5b	48	52	48	50	110	108	108	100	70	70	70	72	24.9	43.5
end	58	58	63	60	100	110	108	100	70	78	74	72	25	40.3
Subject 02	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.192426	3.245511	3.235104	3.112955	93.10622	85.00176	121.5548	130.692	131.5522	1.336849	-2.48936	43.00273	55	57
b2	3.238943	2.87117	3.107025	2.917525	107.4161	64.66118	105.6925	98.45103	97.06926	-4.07286	-9.92354	63.45588	64	63
w2	2.941282	2.744868	3.088905	3.074582	132.246	75.00079	178.1112	150.5203	140.8642	5.019015	4.532051	137.4791	61	59
w3a	3.301651	2.942831	3.415825	3.404445	141.3804	85.51794	178.4486	141.8982	128.6212	3.458077	3.113408	108.668	67	71
w3b	3.097847	3.008084	3.333976	3.278281	130.3505	97.93103	173.1089	142.7588	147.5913	7.62236	5.824505	76.76618	64	72
w4a	3.200964	2.996159	3.339572	3.326707	97.43738	73.96709	185.1737	145.8801	148.1099	4.330173	3.928279	150.3461	59	58
w4b	2.985588	2.934577	3.093306	3.042368	77.77153	57.76284	131.7437	113.293	107.2469	3.607945	1.901823	128.0769	68	67
w5a	3.098572	3.059383	3.289293	3.21587	97.59866	61.03693	115.1809	117.5966	111.2123	6.155102	3.785538	88.70691	62	61
w5b	2.989115	2.920563	3.07484	3.01645	87.77011	68.97112	170.3509	123.1066	123.803	2.867907	0.914512	146.9887	58	50
end	2.83954	2.838262	3.007106	2.953206	78.45782	68.62611	134.494	104.8359	109.8346	5.901155	4.002949	95.98078	65	55
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	57	55	58	61	108	102	109	108	68	68	68	68		
b2	60	68	68	68	118	115	115	112	78	74	75	74	26.33	41
w2	60	60	62	60	112	115	115	115	70	70	70	70	26.2	41.7
w3a	68	69	70	75	117	120	117	117	66	65	65	65		46.3
w3b	64	68	64	68	118	120	118	116	70	64	62	62	26.25	47
w4a	59	60	58	55	102	104	100	104	70	70	72	70		
w4b	63	67	62	62	104	102	108	110	72	72	70	74	26.4	52
w5a	60	56	60	64	102	100	104	104	70	68	70	70	26.25	53
w5b	45	50	47	50	108	100	100	100	70	72	70	70	26.7	53.5
end	60	61	54	60	100	110	100	110	78	74	80	78	26.65	48

Subject 03	ND Left															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.413776	3.532603	3.805356	3.797566	105.3499	98.79823	164.1405	137.9483	127.9347	11.4706	11.2424	66.13705	107	105		
b2	3.552075	3.38935	3.769701	3.661681	96.37962	90.69031	160.3531	120.348	113.2772	6.126725	3.085692	76.81395	70	64		
w2	3.461796	3.608322	3.808833	3.765874	83.96552	73.62392	145.6957	131.9079	138.1116	10.02476	8.783821	97.89181	74	70		
w3a	3.627345	3.551562	3.641691	3.626881	86.89689	77.24176	150.8797	131.2145	129.8297	0.3955	-0.01278	95.33436	61	61		
w3b	3.574459	3.579804	3.65764	3.639789	96.7318	71.38616	173.4702	141.7258	135.1787	2.327087	1.827691	143.0026	70	65		
w4a	3.687038	3.511362	3.856274	3.741588	85.17943	57.41483	144.3278	113.4587	106.7355	4.590007	1.479495	151.3772	68	64		
w4b	3.626511	3.534809	3.656324	3.587947	83.63207	63.45202	139.1467	114.662	105.8649	0.82208	-1.06339	119.2943	65	72		
w5a	3.425454	3.429211	3.61486	3.549917	112.419	77.07554	176.0401	155.0162	150.5293	5.529358	3.633475	128.3994	69	65		
w5b	3.481338	3.507269	3.679396	3.560615	83.80018	64.14021	154.8404	117.9415	108.2773	5.689125	2.277194	141.4093	66	65		
end	3.456144	3.585061	3.70081	3.682087	94.83658	67.93694	146.4	130.706	125.8765	7.079167	6.53742	115.494	64	64		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG		
b1	102	95	92	92	158	156	152	152	60	70	72	68				
b2	67	67	64	63	120	117	117	117	80	80	80	80	25.27	40		
w2	69	70	70	71	120	122	122	118	68	68	68	70	25.3	41.3		
w3a	58	58	56	61	122	120	120	118	66	66	68	68	25.3	39.7		
w3b	67	69	71	68	122	120	120	122	60	60	60	64	25.55	41		
w4a	65	68	63	65	122	118	118	120	78	72	72	74				
w4b	78	82	70	71	118	120	120	128	68	66	68	70	25.2	43.67		
w5a	65	63	62	70	118	120	118	120	76	72	74	74	25.35	45		
w5b	62	63	58	61	120	120	116	122	80	78	76	78	25.7	42.3		
end	61	64	63	67	112	114	120	116	68	70	80	70	25.45	43.33		
Subject 03	D Right															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.525389	3.647262	3.914923	3.882169	116.7282	116.0427	177.0718	149.6572	135.1815	11.04941	10.12032	52.59193	98	91		
b2	3.707897	3.548883	3.96858	3.855526	91.55597	98.29163	178.2854	134.8285	135.0009	7.030478	3.981462	81.38411	80	87		
w2	3.502581	3.594974	3.776981	3.733185	88.62136	88.10772	153.4493	133.4611	135.0035	7.834214	6.583818	74.16096	70	73		
w3a	3.645962	3.524997	3.681373	3.607045	95.86328	77.07041	155.5248	118.1047	113.7976	0.97123	-1.06741	101.7957	59	63		
w3b	3.643301	3.492343	3.713357	3.608055	96.20937	108.4483	175.3697	140.6948	132.9347	1.922868	-0.96744	61.7081	70	64		
w4a	3.91844	3.673479	3.96594	3.933994	93.28001	82.58804	153.6277	130.6936	123.4531	1.212225	0.396945	86.01691	69	68		
w4b	3.618795	3.488536	3.607234	3.569016	79.82909	84.82933	147.0761	120.0062	127.0751	-0.31946	-1.37557	73.37879	79	71		
w5a	3.722325	3.70765	3.758303	3.714383	88.28664	87.94489	165.1782	117.427	114.8379	0.966546	-0.21336	87.82009	77	69		
w5b	3.670674	3.598855	3.734233	3.681956	93.10881	79.48465	170.3452	128.9858	120.8684	1.731534	0.307357	114.3121	61	60		
end	3.684557	3.493534	3.706253	3.569115	108.4497	110.694	160.1787	118.6287	117.2427	0.588842	-3.13313	44.70404	67	66		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG		
b1	91	82	82	84	148	148	152	148	62	68	62	60				
b2	73	68	65	68	120	130	120	126	75	78	80	86	25.6	43		
w2	65	69	68	65	118	120	120	118	68	70	68	68	25.65	41.5		
w3a	60	59	61	62	118	118	116	118	68	68	70	70	25.5	41.67		
w3b	62	65	68	62	120	120	120	118	64	68	70	70	26	43		
w4a	67	68	72	67	118	118	118	116	72	72	72	72				
w4b	71	72	74	73	130	120	120	120	70	70	70	70	25.7	25.2		
w5a	67	65	67	72	122	122	118	116	72	72	72	72	25.75	43.3		
w5b	62	61	62	63	112	108	108	112	78	76	76	74	26.1	41.67		
end	68	67	65	68	118	114	116	118	72	76	78	74	26.15	42.67		

Subject 04	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.118959	3.172761	3.409273	3.43806	78.967	50.00245	142.941	115.5178	112.0742	9.308044	10.23103	185.8681	85	87
b2	3.563943	3.494032	3.869155	3.889772	105.6987	68.96595	167.2508	153.6209	129.4869	8.563867	9.142368	142.5121	77	74
w2	3.140246	#DIV/0!	3.471983	3.42195	98.96942	71.03991	174.3796	131.9022	127.0699	10.56404	8.970738	145.4671	77	75
w3a	3.402562	3.393251	3.760402	3.649714	91.38475	51.04897	147.2481	122.2513	142.6045	10.51679	7.263714	188.4449	75	76
w3b	3.227571	3.162649	3.448946	3.250036	107.0701	64.8285	156.5565	126.9058	121.2119	6.858901	0.696035	141.4933	70	72
w4a	3.445823	3.487752	3.748678	3.425744	104.1471	70.52277	150.8702	131.9006	140.0087	8.78904	-0.5827	113.9312	75	73
w4b	3.460548	3.498822	3.786103	3.703449	107.0815	89.1406	198.1074	133.8095	140.7025	9.407611	7.019167	122.2415	72	73
w5a	2.949729	3.035165	3.420228	3.297182	77.42136	51.21735	121.3912	112.9331	104.1429	15.95057	11.77915	137.0118	74	70
w5b														
end	3.207487	3.440861	3.712531	3.499303	116.9009	74.82798	150.6899	135.1742	135.6919	15.74579	9.097962	101.3817	77	68
	3.117928	3.123629	3.611818	3.531946	96.55727	70.6913	197.6078	156.899	128.801	15.84034	13.27863	179.5362	72	71
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	77	74	83	81	13	142	138	138	70	76	76	78		
b2	72	69	68	67	118	116	112	112	76	74	74	74	26	41
w2	81	74	74	73	130	120	120	118	72	68	68	70	26.1	38
w3a	70	69	68	67	116	114	112	112	80	80	76	78		
w3b	74	70	77	69	118	116	120	122	74	76	80	82		
w4a	68	73	73	75	116	112	112	112	80	82	82	82	26.35	38.3
w4b	77	75	74	77	118	114	116	120	80	80	80	78	26.3	39.6
w5a			76	77	118	116		116	78	80		82	26.6	37.6
w5b														
end	68	68	70	70	116	108	108	106	80	78	78	78	26.45	39
	72	72	64	64	110	110	112	112	78	78	78	80		
Subject 04	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.12138	3.068585	3.523625	3.365213	98.28131	54.14071	156.0345	131.5553	121.5609	12.88679	7.811703	188.2018	79	75
b2	3.473733	3.2913	3.880368	3.676727	115.693	54.48824	184.5874	148.1036	122.0768	11.70597	5.843674	238.7656	73	74
w2	3.263338	3.106655	3.635952	3.366626	112.9316	59.65617	179.5732	139.8331	129.6563	11.41821	3.165103	201.0136	74	73
w3a	3.158691	2.998196	3.527304	3.276441	82.93465	57.76546	155.8692	120.1794	106.5655	11.6698	3.727793	169.8312	65	74
w3b	3.024515	2.956873	3.419847	3.213102	85.53147	62.76047	168.6338	134.4992	130.1818	13.07095	6.235285	168.6943	72	74
w4a	3.124275	2.973794	3.500259	3.45799	76.3851	66.55579	154.8466	141.735	122.9349	12.0343	10.68138	132.6569	74	75
w4b	3.193808	3.031733	3.576828	3.352392	93.28122	76.91243	167.0864	134.4915	127.072	11.9926	4.965383	117.2424	81	77
w5a	3.050884	2.982176	3.439763	3.27602	83.11057	66.7277	175.8771	142.5864	137.2574	12.74646	7.37937	163.5744	72	79
w5b														
end	3.180981	3.054203	3.486723	3.332413	88.28027	65.34881	165.7038	147.5878	122.7708	9.611561	4.760554	153.5681	76	71
	3.143917	2.992685	3.526904	3.452072	74.31355	45.69029	147.9439	139.6573	140.1781	12.18183	9.801647	223.7971	65	75
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	78	76	80	73	142	140	140	138	60	62	64	68		
b2	71	68	67	67	110	108	108	108	70	72	72	72	26.5	42.7
w2	77	76	78	77	118	112	112	112	72	68	70	68	27	39.7
w3a	75		72	68	116	120	120		78	80	78			
w3b	77	73	71	75	116	118	120	120	74	80	82	80		
w4a	68	68	68	72	110	112	110	112	80	82	80	82	27	42.3
w4b	80	80	78	81	120	118	122	120	82	78	80	80	26.65	45.3
w5a	71	74	82	74	118	116	120	118	82	82	82	82	27.05	42
w5b	71	70	71	72	110	108	108	108	84	80	80	80	26.6	40
end	69	67	71	71	112	110	110	110	80	80	80	80		
												</		

Subject 05	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	4.229489	4.00943	4.346631	4.212346	91.55472	80.69565	152.9412	135.3488	116.0389	2.769652	-0.4053	89.52845	52	57
b2	4.185131	4.129519	4.270802	4.209861	93.45082	57.58826	156.9035	133.46	113.7958	2.047053	0.590904	172.4575	51	51
w2	4.322536	4.287863	4.580487	4.469033	71.72961	77.41527	145.6979	112.5951	105.867	5.967569	3.389151	88.203	56	58
w3a	4.293341	4.260544	4.36703	4.34335	74.48319	46.21204	126.7359	114.6639	91.56145	1.71635	1.164816	174.2486	49	50
w3b	4.527506	4.415838	4.586912	4.668966	133.985	106.554	214.1485	168.9769	155.1741	1.312112	3.124455	100.9765	54	55
w4a	4.238683	4.246194	4.674033	4.50459	87.24751	97.4144	179.6569	133.9719	131.3913	10.27089	6.273346	84.42537	49	51
w4b	4.111188	4.131065	4.347292	4.146197	94.83903	74.15476	174.1572	148.6342	146.3885	5.742962	0.851554	134.8565	57	57
w5a	4.296872	4.173358	4.41341	4.243926	72.41911	82.07549	150.7126	118.8017	110.3459	2.712156	-1.23219	83.62679	49	57
w5b	4.325588	4.322964	4.465412	4.412132	86.5562	77.759	129.8468	117.0855	104.6625	3.232492	2.000745	66.98624	64	52
end														
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	55	57	56	56	122	128	128	126	78	78	80	80		
b2	51	52	52	51	122	120	122	122	66	66	66	70	28.7	53
w2	53	55	53	53	124	122	120	118	74	80	74	74	29.35	56
w3a	46	46	45	50	122	124	122	122	78	80	78	78	28.9	55
w3b	53	53	53	55	128	120	120	120	70	70	70	70	29	49.3
w4a	51	48	50	53	128	122	120	122	80	74	74	76	29	53.3
w4b	63	61	61	61	130	128	128	130	78	76	80	80	29.15	53
w5a	53	55	52	53	118	126	130	128	74	74	80	80		
w5b		55	52	51	112	120		120	72	70		70	28.9	50.33
end														
Subject 05	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.995222	4.165752	4.437243	4.35179	84.84102	69.65723	177.088	125.8669	125.866	11.06373	8.924843	154.2277	58	52
b2	4.274066	4.307813	4.458898	4.292569	96.55485	79.4895	159.1455	147.9312	135.8625	4.324506	0.43293	100.2095	52	47
w2	4.249483	4.204651	4.420872	4.441478	#DIV/0!	61.73281	141.5629	123.6216	118.1135	4.033157	4.518079	129.3155	54	51
w3a	4.19498	4.230068	4.330485	4.304823	80.70144	54.83192	134.8294	114.1426	110.5239	3.230153	2.618423	145.8957	49	46
w3b	4.546194	4.499655	4.665738	4.597313	96.72567	76.56164	152.7642	124.4875	122.4157	2.629538	1.124437	99.53098	53	53
w4a	4.288957	4.257687	4.393124	4.325177	85.17959	62.7605	164.6714	132.7631	134.3192	2.428729	0.844508	162.3807	52	52
w4b	4.170812	3.909838	4.28396	4.046099	89.83818	70.52843	143.1053	133.1226	130.0225	2.712853	-2.99012	102.9044	56	54
w5a	4.242529	4.159226	4.363665	4.198016	73.11147	71.39118	146.2279	127.2509	115.5214	2.855267	-1.04922	104.8262	56	56
w5b	4.468926	4.138349	4.362304	4.207931	76.38243	60.52947	207.2427	137.7659	125.1821	-2.38586	-5.84022	242.3831	57	54
end														
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	53	54	55	54	124	122	124	122	78	72	72	72		
b2	46	49	48	47	120	120	120	122	70	68	70	68	29.7	60
w2	52	52	51	56	122	122	120	120	76	74	78	74	29.8	61.3
w3a	47	46	47	47	118	120	118	118	78	80	78	80	29.9	55
w3b	53	54	54	52	118	120	118	118	68	70	68	70	29.7	54.3
w4a	51	50	51	51	124	118	122	120	72	80	72	72	30	56.3
w4b	56	55	58	56	130	128	132	132	80	78	78	80	29.75	58.3
w5a	53	52	54	57	130	130	130	128	80	80	78	82		
w5b	54	56	55	56	122	126	124	122	74	76	76	74	29.65	52.67
end														

Subject 06	ND Left															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.898877	3.942552	3.97452	3.946404	88.28254	61.9028	142.594	122.5884	121.7254	1.94013	1.218995	130.3515	51	64		
b2	3.924416	3.803475	3.964052	3.849589	84.14076	41.38075	115.0186	100.8651	91.04036	1.009985	-1.9067	177.952	58	53		
w2	3.882448	3.866942	4.117833	4.089259	92.24458	71.03999	133.6318	107.9553	112.4234	6.0628	5.326813	88.10789	57	58		
w3a	3.999859	3.953525	4.228885	4.246274	100.3498	50.00295	146.9115	144.8442	142.2485	5.725871	6.160597	193.8057	70	69		
w3b	3.947975	4.014937	4.378111	4.338627	99.66264	90.51858	148.1206	150.5198	143.2793	10.89511	9.895004	63.63553	81	73		
w4a	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!				
w4b	3.733996	3.836875	4.010507	3.930702	121.0345	99.14033	171.5604	147.4172	145.8673	7.405225	5.267984	73.04803	79	81		
w5a	3.977567	3.981505	4.209278	4.212919	116.3839	70.17326	184.8384	159.3111	154.4878	5.825444	5.916998	163.4029	64	60		
w5b	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!				
end	3.942937	3.88888	3.972033	3.875267	91.03779	69.14137	128.9715	113.2834	117.2424	0.737942	-1.71622	86.53308	52	56		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	58	63	59	59	110	112	108	112	64	70	70	70				
b2	57	58	57	54	100	98	100	100	74	68	72	74	28.2	59.3		
w2	58	58	57	57	102	104	104	102	64	62	62	62	28.3	57.5		
w3a	62	67	63	63	112	120	120	116	62	64	64	60	28.3	66		
w3b	75	76	68	77	130	120	118	120	80	70	70	72	28.5	64		
w4a																
w4b	81	82	84	82	120	120	120		60	68	68		28.35	66.67		
w5a					110	112			68	70						
w5b																
end	57	56	60	60	104	104			64	62			27.95			
Subject 06	D Right															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.926731	3.940145	4.067812	4.061053	74.66298	82.24174	134.6664	119.8388	103.7943	3.592831	3.42069	63.74462	65	59		
b2	3.853002	3.941716	3.945213	3.959894	65.86433	51.73154	131.2161	106.9221	103.9729	2.393208	2.774252	153.6481	61	62		
w2	4.046457	4.02074	4.193139	4.157922	75.52232	77.59578	138.4665	121.2178	112.5929	3.624956	2.754634	78.44591	63	59		
w3a	4.237899				87.76194								61	65		
w3b	4.267207	4.225258	4.2853	4.289825	88.9672	68.96595	168.9662	133.452	129.3106	0.424	0.530054	144.9995	77	79		
w4a																
w4b	4.036017	4.085205	4.186993	4.147693	116.3826	99.31186	165.7139	145.1898	136.7252	3.740708	2.766984	66.86218	78	74		
w5a	4.251244	4.126976	4.23764	4.191894	103.2876	69.82972	163.6236	118.2855	112.9352	-0.31999	-1.39605	134.3179	66	68		
w5b																
end	3.912595	3.758481	3.894948	3.968992	78.10652	59.14295	129.8353	101.0375	92.06961	-0.45102	1.441412	119.5279	60	64		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	59	58	55	57	118	110	112	112	72	72	70	70				
b2	58	58	57	58	116	116	116	116	80	78	78	80	28.15	67		
w2	59	58	59	58	108	110	110	110	62	62	68	64	27.95	61.3		
w3a		64	64	62	118	116		118	62	64		62	28.5	67.3		
w3b	75	71	72	74	124	118	120	120	74	74	70	70	28.45	68.3		
w4a																
w4b		76	74	74	116	116			66	66			28.5	70		
w5a					120	118			80	66						
w5b																
end	61	62	61	60	104	108	106	106	66	66	64	64	28.3			

Subject 07	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.017541	2.852451	3.171773	3.196905	96.38178	51.89939	153.2838	133.4598	128.1053	5.111179	5.944071	195.348	59	66
b2	3.121386	3.062039	3.481385	3.544437	112.9331	60.69974	172.4168	144.8425	146.3846	11.53328	13.55329	184.0486	73	75
w2	3.233016	3.021293	3.540715	3.596864	124.1476	94.82789	159.4942	131.2128	122.5872	9.517392	11.25413	68.19328	81	77
w3a	3.159553	3.202581	3.508307	3.545323	99.49271	90.0135	169.3191	138.4487	131.7325	11.03807	12.20962	88.10409	74	74
w3b	3.639685	3.51345	3.962283	3.928784	108.6232	87.2458	167.0821	155.8742	139.836	8.863355	7.942977	91.50733	82	80
w4a	3.13436	2.970193	3.350588	3.392999	141.7243	71.72783	174.1384	154.1471	150.3656	6.898629	8.251737	142.7766	74	76
w4b	3.482447	3.291375	3.686851	3.789995	128.4487	79.83132	167.2431	146.3811	133.4538	5.869534	8.831374	109.4957	86	87
w5a	3.297694	3.082633	3.552644	3.65903	121.2133	78.45473	153.4521	136.5546	135	7.731179	10.95724	95.59323	92	91
w5b	3.133731	3.046871	3.503904	3.452701	102.7601	48.27903	162.0725	131.387	102.9321	11.81252	10.1786	235.6996	79	75
end	3.076828	3.085342	3.482081	3.429398	132.933	97.93346	153.6348	141.3898	141.7293	13.17113	11.45888	56.87669	72	71
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	66	66	65	64	124	128	126	124	74	70	68	66		
w2	74	75	72	70	124	120	120	120	70	70	70	70	25.3	42
w3a	78	75	76	77	116	122	120	120	64	60	64	62	26.05	42.33
w3b	70	68	68	67	128	126	126	124	80	72	72	70	25.7	46
w4a	80	77	73	74	124	128	128	128	74	78	76	70	25.55	45.6
w4b	74	72	68	72	122	124	128	120	64	70	70	64	25.5	45.3
w5a	85	85	85	83	118	122	118	118	74	74	76	68	25.4	47.6
w5b	87	88	83	88	118	124	124	126	78	76	72	70	25.6	49
end	82	84	78	79	120	122	122	124	70	78	80	78	25.45	50
	69	70	66	70	116	116	118	118	70	74	70	70		
Subject 07	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.031733	2.869353	3.320511	3.313513	88.10785	46.3806	160.8812	139.157	114.837	9.525196	9.294363	246.8718	64	67
b2	3.113476	2.986528	3.48549	3.322962	90.70313	96.20751	154.8285	107.5889	117.4219	11.94852	6.728374	60.93185	74	73
w2	3.023768	2.994939	3.405486	3.408182	122.9515	87.24313	153.4624	143.969	125.1854	12.6239	12.71307	75.90196	80	80
w3a	3.293038	3.200628	3.548814	3.668797	81.89692	83.10379	145.8673	122.0739	125.6927	7.767171	11.4107	75.52421	69	74
w3b	3.661041	3.632121	3.942047	3.870629	111.3859	97.93578	159.3195	145.3567	139.3146	7.675575	5.72482	62.67757	81	76
w4a	3.145393		3.35239	3.393508	131.2098	68.27799	167.2458	147.5945	153.6449	6.580974	7.888209	144.9484	74	71
w4b	3.384329	3.060953	3.488122	3.512108	112.7663	66.21139	169.6724	146.5568	140.3509	3.066872	3.775612	156.2586	84	85
w5a													87	89
w5b	3.333129	3.224052	3.532607	3.510712	92.7628	85.69035	163.1054	141.209	132.0726	5.984689	5.327818	90.34281	78	83
end	3.283876	3.127172	3.504567	3.478666	103.7959	47.2445	148.9681	133.2875	135.6905	6.720441	5.931706	215.3132	77	73
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre180	SBPpre30	SBP60	DBPbase	DBPpre180	DBPpre30	DBP60	forecirc	HG
b1	70	67	64	66	120	124	124	120	76	80	78	76		
b2	77	87	81	75	120	124	124	130	70	74	74	80	27.3	43
w2	77	77	77	77	116	118	116	112	74	76	76	76	26.85	45.67
w3a	68	66	64	64	120	120	120	120	72	74	72	74	27.4	46.7
w3b	83	77	74	77	130	126	124	128	78	80	80	78	26.8	52
w4a	77	73	72	70	122	124	120	120	74	74	72	72	26.8	52.67
w4b	84	82	86	84	120	118	120	122	72	70	84	76	27.2	54
w5a	92	84	83	85	120	120	130	122	76	80	82	76	26.7	54.67
w5b	80	81	79	75	122	120	124	120	76	80	80	80		
end	73	67	75	70	114	114	118	118	78	78	78	76		

Subject 08	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.355653	3.349745	3.69954	3.606386	99.66259	81.3829	182.0858	133.2781	131.5639	10.24801	7.471974	123.7397	71	64
b2	3.414842	3.234767	3.516277	3.494409	91.3835	63.62531	156.2206	136.9003	126.556	2.970432	2.330061	145.5322	58	57
w2	3.620999	3.623525	3.981872	3.910346	82.24176	70.17785	160.0137	121.2157	114.321	9.96612	7.990805	128.0117	64	62
w3a	3.495374	3.550078	3.936402	3.951058	108.6221	87.9324	186.8998	143.4491	143.4503	12.61749	13.03677	112.5494	79	79
w3b	3.673492	3.595451	3.975741	3.959637	114.3155	96.90052	249.3299	146.5558	130.1803	8.227831	7.789445	157.305	78	77
w4a	3.441617	3.386334	3.973145	4.010913	112.5896	96.38083	200.5217	142.9352	125.7003	15.44415	16.54153	108.0514	80	86
w4b	3.513394	3.52541	3.940532	3.846434	100.1801	82.42279	181.56	138.2814	118.4503	12.15743	9.479176	120.2789	70	70
w5a	3.482073	3.434201	3.91336	3.879164	92.2417	99.1394	195.179	127.4233	126.7363	12.38594	11.40388	96.87326	68	69
w5b	3.491625	3.519952	4.068026	4.00106	108.9735	99.3142	198.9734	159.8393	155.6953	16.5081	14.59021	100.3473	65	69
end	3.53732	3.558059	3.90934	3.887497	91.72575	56.73106	154.6648	131.3815	115.6922	10.517	9.899503	172.628	58	59
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	62	63	57	62	122	128	122	124	74	72	70	76		
b2	58	57	59	59	122	124	122	122	80	74	74	76	28.2	44.6
w2	64	60	63	64	130	134	134	136	80	82	80	80	28.3	37.7
w3a	80	82	99	90	132	138	140	144	84	80	84	82	28.2	44.3
w3b	81	73	79	82	146	140	138	138	84	82	82	82	28.05	41.3
w4a	78	75	75	76	148	146	138	140	86	84	78	78	28.3	46.3
w4b	68	68	74	73	122	128	124	130	76	76	68	76		
w5a	73	74	76	72	130	126	130	130	68	76	78	78	28.15	47.3
w5b	64	61	62	67	132	130	130	128	74	72	70	70	28.6	45.67
end	60	59	58	60	124	124	126	126	74	78	72	72		
Subject 08	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.422925	3.424337	3.767479	3.734184	84.3175	79.3122	171.2353	112.9313	105.1815	10.06607	9.093365	115.9003	65	63
b2	3.441171	3.326883	3.7798	3.593543	74.66792	80.18428	189.6695	115.1788	115.7044	9.840492	4.427908	136.542	60	68
w2	3.701039	3.719096	4.085902	4.038411	96.5557	65.86967	182.0886	127.2456	117.7612	10.39878	9.115615	176.4377	63	59
w3a	3.609599	3.757446	3.976753	3.916216	111.386	99.65786	203.9746	150.5347	142.7621	10.17161	8.494501	104.6749	85	79
w3b	3.652414	3.695472	3.945095	3.885619	104.6577	88.97155	153.1044	114.3116	120.6928	8.013349	6.384931	72.08246	78	67
w4a	3.727332	3.593258	3.918397	3.798634	103.7989	89.48828	171.0413	135.1747	116.3798	5.126055	1.912941	91.13266	78	69
w4b	3.485672	3.496206	3.805038	3.761399	106.2174	84.31495	195.3558	134.6574	125.0059	9.162238	7.910306	131.6977	74	66
w5a	3.561181	3.445695	3.669418	3.714605	93.28903	81.03448	160.1766	118.2853	107.7642	3.039381	4.30825	97.66472	79	74
w5b	3.703697	3.637072	4.035363	3.941524	112.0779	85.6959	205.6986	146.2162	149.1454	8.95498	6.421334	140.0331	61	66
end	3.787033	3.543846	3.903591	3.889249	72.07692	72.93469	175.1869	119.3215	109.1434	3.07782	2.699117	140.1969	65	60
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	67	64	58	61	122	124	126	124	80	78	80	78		
b2	65	63	85	66	120	122	130	128	82	82	80	80	29.2	48
w2	60	59	60	58	133	140	138	140	82	86	84	88	28.85	49.7
w3a	73	73	74	72	144	140	140	130	82	80	80	80	29.1	53.67
w3b	70	67	71	72	142	132	130	130	84	82	80	78	29.3	53.3
w4a	77	65	74	75	140	134	130	130	82	80	82	80	29.05	52.67
w4b	69	67	68	71	128	124	120	120	80	76	78	76		
w5a	67	72	68	73	130	134	126	126	84	78	74	68	29.15	52.67
w5b	60	62	61		124	130	124	124	70	74	70	72	29.05	52.33
end	70	64	65	67	126	122	122	122	76	74	78	78		

Subject 10	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	2.659333	2.654125	2.873712	2.893593	58.46455	37.58699	145.3465	95.0052	93.28219	8.061381	8.808969	286.6935	64	68
b2	2.753258	2.730101	2.975324	2.897133	60.86444	35.17675	138.2797	88.97397	92.0719	8.065581	5.225627	293.0998	76	69
w2	2.871514	2.768477	3.05425	3.030065	50.86497	38.79701	147.9379	98.10869	92.06929	6.363751	5.521518	281.3125	76	74
w3a	2.848709		3.157949	3.15372	72.41461		135.6952	105.8716	94.6558	10.85541	10.70698		76	80
w3b	2.975431	2.890033	3.306803	3.256169	78.44864	47.24138	146.3862	109.8325	106.3843	11.13695	9.435197	209.8687	82	83
w4a	2.907725	2.852902	3.150099	3.067344	67.24183	57.07001	144.8303	118.9735	111.2104	8.335501	5.489449	153.7765	79	84
w4b	3.015176	2.811311	3.27976	3.217907	77.76246	48.10583	163.4565	126.9083	121.9035	8.775086	6.72369	239.7852	68	72
w5a	2.977874				68.625	44.13928							77	76
w5b	3.045082	3.03459	3.482504	3.393462	66.731	48.62133	148.2778	99.48814	89.66114	14.36487	11.44074	204.9646	73	79
end	2.877541	2.831573	3.27702	3.221991	62.93198	35.00087	136.208	109.139	91.041	13.88266	11.97029	289.156	72	70
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	73	73	70	72	110	108	108	106	80	78	72	72		
b2	77	76	70	78	100	102	98	102	72	80	76	76	24.8	45.3
w2	76	71	73	72	110	112	112	108	70	68	68	64	25.1	44.6
w3a	76	77	75	77	102	108	106	108	76	78	80	74	25	40.6
w3b	82	85	82	82	102	98	100	98	72	76	74	70	24.9	44.67
w4a	78	82	80	82	102	100	100	100	72	70	70	70		
w4b	74	71	76	78	98	100	98	102	68	74	70	74	25.75	42.7
w5a	75	73	72	74	98	98	98	96	72	72	70	72	25	46.3
w5b	77	76	77	75	96	96	96	96	68	68	70	70	25.05	46
end	70	71	72	74	100	96	94	98	76	72	70	70		
Subject 10	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	2.679929	2.599597	2.836021	2.814544	73.44976	65.5191	167.7596	107.4174	100.0003	5.824464	5.023089	156.0469	76	78
b2	2.771642	2.644395	2.948511	2.934163	72.59638	62.24326	151.0394	115.3512	106.7255	6.381393	5.863716	142.6598	78	72
w2	2.943447	2.670737	2.847957	2.79592	65.17606	90.8637	145.1856	101.73	101.5638	-3.24417	-5.01206	59.78395	76	70
w3a	2.927802	2.785048	2.958632	2.850353	72.58703	88.97388	180.364	136.559	127.42	1.052997	-2.64529	102.7157	74	78
w3b	2.878187	2.8232	3.107461	3.030741	85.00589	75.00994	154.3325	134.6608	120.6909	7.965933	5.300368	105.7494	85	82
w4a	2.960863	2.755506	2.955514	2.912983	82.93566	77.58697	149.6578	113.7957	107.9321	-0.18065	-1.61707	92.89035	76	76
w4b	2.831545	2.684607	3.132344	3.075377	78.62438	88.28123	158.4537	117.2541	113.626	10.62312	8.611246	79.48742	75	74
w5a	2.782737	2.75823	3.042297	2.924247	80.1791	92.5894	170.1837	121.7285	106.035	9.327501	5.08528	83.80472	75	73
w5b	2.892958	2.921402	3.216584	3.261753	66.03896	72.76681	139.1422	109.663	103.7979	11.18667	12.74803	91.21656	71	75
end	2.851355	2.748407	3.126714	2.982996	64.14217	64.48747	166.5521	113.9744	103.6221	9.657137	4.616789	158.2704	70	71
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	69	73	74	73	104	108	108	104	70	76	78	74		
b2	72	76	77	77	102	102	108	102	74	76	78	78	24.55	44
w2	78	70	70	76	110	110	112	110	72	76	70	70	24.7	47
w3a	79	77	78	76	110	112	106	110	78	78	76	78	24.55	48.33
w3b	78	82	82	79	98	98	102	98	78	76	76	74	24.5	48.33
w4a	77	80	78	77	102	104	100	102	72	74	70	72		
w4b	73	73	71	74	100	98	100	98	72	70	72	72	24.65	47
w5a	75	69	73	72	110	96	98	100	78	72	72	72	24.9	45.67
w5b	78	72	75	74	100	98	98	98	72	70	74	74	24.55	46
end	70	70	71	72	102	100	94	102	72	72	72	74		

Subject 12	ND Left															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	2.865834	2.953333	3.048545	3.015281	73.79843	56.72414	137.0759	102.0696	104.6557	6.375464	5.214783	141.6536	70	73		
b2	2.768345	2.904707	2.965901	2.948863	104.4828	78.62439	143.636	112.94	102.0704	7.136255	6.52079	82.68633	75	69		
w2	2.836097	2.932417	2.958313	2.957754	82.93695	73.97563	147.939	119.8278	115.1751	4.309307	4.289601	99.98339	64	64		
w3a	2.96444	2.987062	3.140477	3.217699	105.5228	95.69493	152.2634	124.6583	126.8996	5.938268	8.54323	59.11336	74	77		
w3b	2.931274	2.919054	3.113595	3.169108	83.79381	91.03776	150.6971	113.11	105.6911	6.219878	8.113685	65.53249	62	73		
w4a	2.777629	2.646755	3.009417	2.936034	73.45348	58.97031	133.6298	100.1739	106.7255	8.344846	5.702919	126.6052	61	65		
w4b	2.864291	2.887275	3.192505	3.131366	93.454	57.75914	137.0852	112.5896	110.357	11.45882	9.324295	137.3395	62	66		
w5a	2.828477	2.956099	2.973401	2.949536	82.06969	58.62069	148.1044	109.4833	107.0833	5.12376	4.280029	152.6488	65	65		
w5b	2.855849	2.890186	3.035273	3.098439	87.93947	60.00192	144.4894	116.8988	113.2843	6.282696	8.494512	140.8079	72	68		
end	3.079586	3.094036	3.346801	3.296024	78.28348	68.45389	134.8333	107.4149	103.9692	8.677003	7.028161	96.96944	70	69		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	71	75	76	73	114	120	120	122	88	90	88	90				
b2	72	72	73	72	110	118	116	118	68	80	80	80	24.5	27		
w2	65	64	64	67	110	112	108	108	72	70	72	74	24.9	26		
w3a	78	76	75	76	120	112	116	118	74	72	72	74				
w3b	63	61	61	64	120	118	118	116	84	84	84	84	24.7			
w4a	64	60	63	65	104	104	118	116	84	88	84	84	24.4	25.3		
w4b	62	64	62	65	112	116	110	110	78	82	82	82	24.55	24.3		
w5a	68	65	65	66	110	108	112	110	82	82	84	84	24.75	27.6		
w5b	64	68	68	71	116	108	112	110	78	76	80	80	24.55	28.3		
end	67	68	70	73	114	118	110	108	72	80	82	78				
Subject 12	D Right															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.299001	3.183216	3.298045	3.216848	63.10393	92.76277	111.7252	89.48445	80.17425	-0.02897	-2.49024	20.44188	75	82		
b2	3.43453	3.399872	3.449276	3.313743	61.729	64.31215	110.1752	81.03922	79.13869	0.429359	-3.51685	71.31318	79	80		
w2	3.3905	3.343968	3.459771	3.482314	75.34859	76.38129	154.3246	112.2466	99.31156	2.043083	2.707962	102.045	64	64		
w3a	3.496358	3.402628	3.605809	3.468149	91.72544	65.86252	136.558	117.9442	112.7613	3.13042	-0.80681	107.338	77	77		
w3b	3.501323	3.425333	3.449633	3.426886	73.79511	85.0007	140.0004	95.51882	86.20759	-1.47629	-2.12596	64.70503	60	60		
w4a	3.269614	3.258558	3.332955	3.387607	69.13879	94.3136	150.0148	108.6261	94.49057	1.937261	3.608778	59.05952	62	63		
w4b	3.530054	3.527716	3.454641	3.446677	68.10911	62.93575	116.3798	103.1058	91.21504	-2.1363	-2.3619	84.91847	63	65		
w5a	3.283144	3.255705	3.478335	3.34327	68.79525	53.27586	109.8303	85.17828	76.55484	5.945238	1.831355	106.154	66	65		
w5b	3.557771	3.343933	3.487613	3.419469	70.86934	69.65731	139.6167	106.217	99.66054	-1.97196	-3.88732	100.4337	67	67		
end	3.613128	3.406517	3.592931	3.524854	78.62218	62.58669	145.6974	96.90057	99.14548	-0.55898	-2.44316	132.793	70	70		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	78	75	79	74	116	118	118	118	88	86	86	88				
b2	71	76	69	75	118	116	116	116	80	80	80	80	25.2	29		
w2	67	64	61	67	106	104	108	106	78	72	76	74	25.4	29.6		
w3a	78	71	73	77	112	110	114	112	72	74	74	72				
w3b	62	61	62	65	112	116	110	110	84	80	80	82	25.15			
w4a	71	68	62	64	110	112	116	110	80	78	82	80	25.25	22		
w4b	63	66	68	65	108	108	110	112	78	82	82	84	25.4	24.3		
w5a	64	75	66	76	108	104	108	112	80	84	82	84	25.1	30		
w5b	66	73	68	75	104	108	108	110	80	78	80	80	25.1	27		
end	70	68	70	75	114	108	106	116	80	80	80	84				

Subject 13	ND Left													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.126419	3.033025	3.346116	3.343945	141.7277	98.27981	204.3304	154.4837	144.3229	7.027101	6.957669	107.9068	80	77
b2	3.067688	3.019984	3.190687	3.181611	117.4268	68.27935	165.0094	133.6271	117.7586	4.009509	3.713631	141.6682	71	71
w2	3.458904	3.423479	3.617909	3.542736	98.10647	75.52433	164.6813	132.7624	121.3805	4.596998	2.423665	118.0506	63	61
w3a	3.335179	3.232851	3.508515	3.428759	137.4159	65.51922	140.6917	127.5902	126.5536	5.197215	2.805852	114.7335	66	67
w3b														
w4a	3.186631	3.01126	3.444005	3.408488	153.2797	114.487	220.0114	175.1865	171.0388	8.076697	6.962124	92.17153	73	77
w4b	3.222601	3.094667	3.453107	3.426341	107.9324	80.52645	192.419	144.8303	151.566	7.152798	6.322226	138.9513	80	76
w5a	3.257061	3.111355	3.454243	3.515649	108.7942	85.0028	179.4862	136.5545	131.2071	6.054004	7.939311	111.1533	82	77
w5b	3.37453	3.220792	3.537989	3.478365	107.5951	72.06979	176.8998	146.0347	141.2107	4.8439	3.077023	145.4562	67	77
end	3.187492	3.095528	3.348299	3.28113	106.2075	85.00305	194.4861	155.0025	153.7996	5.044926	2.937659	128.799	62	62
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	79	76	74	76	116	112	110	110	64	68	64	64	25.5	43
b2	78	71	72	70	110	110	114	108	68	68	70	68	25.6	47.6
w2	63	63	63	64	102	100	102	100	60	64	64	62	25.75	48
w3a	67	68	69	65	112	106	106	104	64	66	64	64	25.7	50.3
w3b														
w4a	76	75	73	73	112	108	110	106	58	58	58	58	25.55	49
w4b	79	75	74	77	106	104	110	104	60	62	62	64	25.65	57.67
w5a	73	72	74	80	102	100	98	110	64	64	62	70	25.45	55
w5b	69	67	66	68	96	100	100	100	62	68	68	68	25.45	54.33
end	61	63	61	60	100	104	104	102	62	64	64	64		
Subject 13	D Right													
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.334545	3.243795	3.539803	3.56598	123.2868	82.75898	198.4542	129.8369	132.5993	6.155508	6.940527	139.7978	77	84
b2	3.120823	3.122308	3.399029	3.320114	100.3478	65.51724	162.0733	130.8632	124.8314	8.914488	6.385841	147.3751	72	79
w2	3.298185	3.331967	3.4225	3.376426	86.38	79.31222	155.7113	117.2545	110.8687	3.769185	2.372241	96.32698	68	60
w3a	3.450787	3.521838	3.672068	3.602443	101.9051	75.34676	165.5415	136.7252	131.0483	6.412491	4.394821	119.7062	69	65
w3b														
w4a	3.308294	3.32022	3.578044	3.524777	138.2871	89.83893	200.3532	151.0365	155.0033	8.153721	6.543625	123.0138	72	77
w4b	3.23153	3.260848	3.549315	3.503297	119.6562	91.55469	171.0537	141.7252	133.6216	9.83388	8.409846	86.83222	70	75
w5a	3.48426	3.409317	3.663963	3.630879	102.2496	100.692	160.3624	137.7641	138.9711	5.157567	4.208034	59.26026	70	74
w5b	3.380558	3.43956	3.572255	3.528833	97.58932	64.48878	146.3911	120.5234	112.2414	5.670561	4.386107	127.0025	74	68
end	3.250404	3.135396	3.333903	3.285006	89.14369	63.62303	153.1114	137.414	117.5912	2.568883	1.064553	140.6541	64	61
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	78	76	75	77	110	112	110	110	68	70	68	68	26.3	47.67
b2	71	76	82	79	112	114	112	114	68	68	68	70	26.3	48.33
w2	62	60	59	57	108	106	108	104	72	72	70	70	26.5	52.33
w3a	66	64	64	63	108	106	108	108	68	70	68	68	26.7	52.33
w3b														
w4a	74	75	73	76	102	112	108	108	60	60	60	62	26.3	53
w4b	76	76	73	71	106	100	102	104	62	60	64	64	26.1	62
w5a	69	69	68	65	106	104	102	100	66	66	68	62	26	60.67
w5b	66	66	67	64	100	96	96	92	68	66	68	68	26.2	58
end	59	58	60	58	102	102	106	104	70	78	68	68		

Subject 14	ND Left													
	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	2.786079	2.786639	2.932946	2.905556	67.76442	73.9659	122.5906	100.6902	88.11314	5.271447	4.288352	65.73932	62	65
b2	2.863772	2.878802	2.846497	2.876953	68.99181	80.52353	123.1158	95.52127	79.48667	-0.60324	0.460266	52.89419	48	49
w2	2.843804	2.920327	3.040122	2.923699	56.2074	70.51724	133.2826	106.0356	97.58651	6.90336	2.809464	89.00705	72	58
w3a	2.761133	2.869934	3.126056	3.12748	72.94304	65.86996	148.2955	114.3209	109.6598	13.21642	13.268	125.1337	63	63
w3b	2.73368	2.774755	3.047159	2.991806	58.45478	65.00445	150.0099	101.7299	73.10877	11.46731	9.442461	130.7687	65	60
w4a	2.667493	2.580111	2.97188	2.987	71.21105	79.48351	136.041	102.946	84.83113	11.41099	11.97779	71.15628	69	66
w4b	2.888899	2.885308	2.991424	3.04452	68.62156	61.8985	123.8042	101.3805	95.52636	3.548947	5.386851	100.0117	58	62
w5a	2.910296	2.90283	3.110488	3.000729	55.17349	43.9662	117.0723	92.59258	105.0023	6.878759	3.107339	166.2779	55	58
w5b														
end	2.839477	2.701831	3.051222	2.975801	82.76588	88.62411	130.0167	113.2793	100.5246	7.457192	4.801033	46.70582	59	58
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	65	67	72	68	100	100	102	102	70	70	70	70		
b2	51	50	51	52	100	104	104	100	64	70	64	64	22.7	36
w2	62	61	60	62	94	90	94	92	70	72	70	72	22.85	35.33
w3a	63	63	63	63	108	102	104	102	70	72	72	72		
w3b	70	68	67	64	120	116	116	116	88	84	84	86	22.9	36.67
w4a	68	67	66	66	100	104	104	102	66	72	72	70	22.75	36.7
w4b	60	65	65	73	110	106	106	106	76	78	76	76		
w5a	58	59	58	61	98	100	100	98	68	66	66	66	22.9	36.7
w5b														
end	59	64	62	62	114	104	104	100	80	72	74	72		
Subject 14	D Right													
	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.003932	2.985095	3.083056	3.065155	67.93493	75.17906	131.73	98.11899	96.73878	2.633994	2.038103	75.22172	67	77
b2	3.05008	3.01058	3.013882	3.031364	53.62069	59.48482	98.44978	80.69344	70.34567	-1.18682	-0.61364	65.50404	55	58
w2	3.138298	3.029446	3.082954	3.163858	64.48463	82.76326	141.5545	109.3224	103.4555	-1.7635	0.814457	71.03539	61	59
w3a	3.022985	2.957016	3.050201	2.99794	73.45232	77.24519	123.2941	106.8966	97.41688	0.900295	-0.82848	59.61392	63	60
w3b	3.041835	2.960595	3.158454	3.099954	65.00092	93.97066	143.2761	104.4877	94.65928	3.833842	1.910667	52.46894	65	62
w4a	3.10395	3.126536	3.329665	3.263111	62.58668	73.11077	119.4898	94.66439	77.9318	7.271853	5.127703	63.43661	65	62
w4b	3.023035	2.858985	2.951285	2.840408	72.58823	75.86556	145.0059	107.5868	87.07345	-2.37344	-6.04121	91.13531	67	66
w5a	3.077322	3.151396	3.357365	3.339021	56.20953	60.00203	102.5876	88.28193	81.37967	9.100201	8.504118	70.97357	64	60
w5b														
end	3.171547	3.115045	3.358077	3.318828	66.55976	66.72592	118.627	92.59421	89.14131	5.881348	4.643806	77.78253	66	62
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	69	74	67	65	102	102	104	104	74	72	72	70		
b2	53	56	52	56	104	104	104	104	70	70	68	70	23.55	36
w2	60	62	61	62	94	96	96	96	70	78	76	76	23	32.67
w3a	62	62	62	58	110	106	106	104	74	70	72	74		
w3b	67	65	63	65	112	116	114	118	84	80	82	80	23.75	36.67
w4a	67	64	64	62	108	108	106	108	72	72	70	70	23.55	36.5
w4b	66	62	64	57	106	106	104	106	76	78	78	70		
w5a	63	62	60	63	100	98	98	100	68	68	66	70	23.35	36
w5b														
end	59	68	62	68	106	108	112	106	78	78	78	80		

Subject 15	ND Left															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.589853	3.60801	3.905656	3.895725	86.72448	61.21185	175.702	116.3806	103.803	8.797081	8.520461	187.0391	60	63		
b2	3.680862	3.894343	3.914172	3.725727	95.17368	57.24553	131.561	110.3483	103.7934	6.338443	1.218859	129.8187	64	61		
w2	4.035264	4.018634	4.381597	4.373604	67.93834	36.72414	157.4371	115.1757	100.8644	8.582654	8.384574	328.7021	60	67		
w3a	3.84181	#DIV/0!	4.413302	4.09257	97.76136	45.6903	193.9934	116.3865	107.2425	14.87562	6.527139	324.5834	71	62		
w3b													61	77		
w4a	4.139293	3.96464	4.351011	4.206526	90.34807	38.79386	155.0004	115.5224	118.7973	5.114814	1.624246	299.5488	70	70		
w4b	3.838151	3.676389	4.011766	3.967943	80.34779	54.48489	135.8657	104.4902	92.25454	4.523412	3.381621	149.3641	68	61		
w5a	3.927995	3.948686	4.193444	4.191848	83.79913	27.24357	151.215	114.8367	107.5906	6.757877	6.717248	455.0484	66	63		
w5b	3.958974	4.048415	4.517087	4.176301	91.38741	61.72414	156.3903	116.8968	101.7256	14.09741	5.489492	153.3698	69	67		
end	3.675044	3.439266	3.876415	3.761365	66.37976	35.86294	176.0407	114.3149	102.5891	5.479394	2.348835	390.8709	52	57		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	62	67	61	63	122	120	118	112	60	60	60	60				
b2	63	64	67	65	106	108	104	108	60	60	68	70	24.8	29		
w2	70	71	70	71	110	116	116	116	70	74	74	74				
w3a	65	62	58	63	116	108	110	104	72	72	72	68	24.5	39		
w3b	72	66	68		112	114			70	80						
w4a	67	66	68	70	106	102	106	108	70	70	68	66	24.75	38.3		
w4b	63	61	62	60	106	100	110	110	74	74	70	70	24.9	41.7		
w5a	64	64	62	62	108	98	98	100	72	64	70	70	24.85			
w5b	61	64	68	66	112	104	106	106	68	66	72	70	24.6	38.33		
end	51	53	54	51	108	112	112	116	84	80	82	80				
Subject 15	D Right															
	diambase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180		
b1	3.882378	3.407116	3.697833	3.593282	78.28338	50.69083	171.0348	106.3807	88.11181	-4.75339	-7.44635	237.4078	59	62		
b2	3.794989	3.707679	3.845827	3.783032	77.94055	83.79381	164.147	120.5175	102.0742	1.339621	-0.31505	95.89392	70	68		
w2	3.665825	3.353971	3.753846	3.549544	97.58923	47.936	157.4259	106.0418	101.5618	2.401142	-3.17203	228.4086	81	71		
w3a	3.962651	3.680661	3.965715	3.762247	94.31346	62.42237	154.4835	101.036	113.7944	0.077324	-5.05731	147.481	61	64		
w3b													72	69		
w4a	4.121513	3.843866	4.058003	4.026973	89.65584	61.89855	174.4858	115.8646	97.59014	-1.54095	-2.29381	181.89	73	78		
w4b	3.840727	3.753099	4.082676	4.011258	92.07221	51.89952	144.3145	95.52001	98.80317	6.299584	4.440089	178.0651	65	59		
w5a	3.937819	3.767049	4.108304	4.049118	65.52313	35.52145	154.8317	112.2427	106.3818	4.329427	2.826418	335.8823	61	63		
w5b	3.594283	3.379698	4.097222	3.984644	87.24206	54.31269	176.3815	120.8671	104.1451	13.99274	10.8606	224.7519	84	72		
end	3.636294	3.566296	3.903993	3.787244	71.38096	50.0051	139.8315	109.8289	91.38737	7.361859	4.151225	179.6344	61	56		
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG		
b1	71	62	61	72	106	110	110	110	70	68	70	70				
b2	64	58	67	64	100	100	102	98	70	70	70	70	25.7	34.67		
w2	65	68	67	65	104	110	106	104	82	80	82	82				
w3a	58	57	66	70	100	100	100	104	70	68	70	74	25.55	38.67		
w3b	81	61	78	62	106	108			78	78						
w4a	65	67	69	72	100	100	100	104	72	70	70	70	25.8	41		
w4b	57	62	61	61	106	102	104	102	76	68	72	66	25.65	43		
w5a	65	62	61	63	98	98	98	96	72	68	72	70	25.9			
w5b	68	64	70	71	112	106	106	104	70	74	72	74	25.65	44.67		
end	52	52	52	54	110	112	100	108	84	84	84	80				

Subject 16	ND Left													
	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.32406	3.274896	3.700142	3.478681	117.2502	114.8328	175.5353	135.3574	127.9332	11.31395	4.651565	52.86171	60	61
b2	3.448636	3.533701	3.875009	3.725923	116.9028	123.9703	191.065	151.212	141.0398	12.36355	8.040477	54.12162	68	65
w2	3.475787	3.419624	3.92513	3.705724	119.6564	101.0375	164.8512	128.4541	127.4168	12.9278	6.61539	63.1584	68	64
w3a	3.447038	3.560535	4.154752	4.03577	123.9778	103.9669	156.3909	131.3811	121.3906	20.53107	17.07934	50.42369	73	72
w3b	3.43366		4.123752	3.858136	124.489	130.5209	194.3312	158.7978	139.4896	20.09786	12.3622	48.88898	67	67
w4a	3.547751	3.467939	3.910365	3.631957	126.897	109.8471	175.3668	141.56	132.7622	10.22096	2.373484	59.64629	65	64
w4b	3.460382	3.405741	4.165597	4.014922	126.8987	89.49965	205.7467	166.3896	145.8655	20.37971	16.02543	129.8855	70	66
w5a	3.538087	3.310165	4.07286	4.129832	115.3474	64.65749	154.1404	146.3812	129.1473	15.11475	16.725	138.3953	67	69
w5b														
end	3.456018	3.386308	3.765655	3.729818	115.0003	111.2079	173.4487	133.1037	121.2074	8.959343	7.922411	55.96791	62	61
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	61	61	64	60	112	114	110	110	70	72	70	70		
b2	67	67	65	67	112	112	110	110	62	64	70	70	26.2	42.6
w2	61	62	63	64	110	114	108	110	68	70	68	70	26.55	34
w3a	68	68	65	66	120	118	112	108	72	72	72	70	26.25	38
w3b	67	69	68	76	122	110	110	110	64	60	60	60	26.75	44.6
w4a	60	63	64	63	120	110	106	110	68	70	68	66		
w4b	68	65	70	67	110	110	110	110	70	70	70	70	27.15	38.3
w5a	71	68	70	69	106	114	114	114	64	66	70	68	27.25	34.6
w5b														
end	62	64	62	62	116	116	114	114	70	70	70	70		
Subject 16	D Right													
	diabase	diampre	diam60	diam90	velbase	velpre	velrel	vel60	vel90	basev60	basev90	prev/relv	Hrbase	HRpre180
b1	3.466095	3.284462	3.491444	3.303866	78.1103	86.21618	152.5991	110.5199	98.10841	0.731324	-4.68046	76.99591	60	58
b2	3.601851	3.35575	3.653288	3.45581	87.24993	87.93137	163.9746	132.4195	128.7973	1.428084	-4.0546	86.48019	64	64
w2	3.758476			3.536919	95.87108				108.4505		-5.89487		56	59
w3a	3.811275	3.677658	3.677503	3.499341	92.41921	92.76439	154.8311	109.6731	111.3865	-3.5099	-8.18452	66.90785	68	73
w3b	3.752567	3.612544	3.731299	3.769604	116.2102	106.3804	145.8741	116.3826	114.4848	-0.56675	0.454017	37.12497	71	69
w4a	3.628568	3.381354	3.909257	3.690542	100.6911	112.9336	157.0932	137.2512	151.0488	7.73552	1.707947	39.10226	61	80
w4b	3.736951	3.512594	3.92534	3.703065	97.41531	83.10805	150.8732	125.5196	109.8321	5.04124	-0.90677	81.53857	74	67
w5a	3.738849	3.621449	4.140314	4.222019	92.42409	97.58771	174.8345	136.7294	125.6897	10.73766	12.92294	79.15632	71	71
w5b														
end	3.60741	3.61631	4.194567	3.8931	84.14146	85.86276	173.97	118.9688	116.3875	16.27641	7.919533	102.614	61	62
	HRpre30	Hrrel	HR60	HR90	SBPbase	SBPpre18	SBPpre30	SBP60	DBPbase	DBPpre18	DBPpre30	DBP60	forecirc	HG
b1	64	59	64	64	110	112	114	112	72	74	74	72		
b2	64	68	68	68	110	110	110	110	68	70	68	68	26.5	45
w2	61	73	65	70	110	114	116	112	72	72	72	72	27	33
w3a	61	65	95	72	110	116	110	120	66	76	70	78	27	36
w3b	70	71	68	74	110	112	110	110	74	68	68	70	27.56	42.3
w4a	68	66	68	72	108	120	120	120	68	84	70	72		
w4b	68	70	67	74	112	110	112	110	70	70	70	68	27.45	41.33
w5a	71	68	72	68	110	108	110	106	78	68	68	68	28	42
w5b														
end	62	65	65	64	114	116	118	116	72	72	74	74		

APPENDIX D. REPRINT LETTER OF PERMISSION

FCR/grd/nov01.008
06 November 2001

Dr Jason Allen
Duke University Medical Center
DUMC 3022
Durham, NC 27710
USA

Dear Dr Allen

AMERICAN JOURNAL OF CARDIOLOGY, Vol 86, No 6, 2000, pp 703-705, Allen et al:
"Influence of age and normal plasma..."

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Yours sincerely

Frances Rothwell (Mrs)
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VITA

Jason David Allen was born June 7th 1970 in the small village of Mapplewell, near Barnsley, in the heart of the Yorkshire mining country, as the only child to David and Ruth Allen. He attended Darton High School and gaining 8 'O' level qualifications, followed by the adjoining sixth form department where he obtained 4 'A' levels. He made his career choice of exercise physiology quite easily, simply following his love for sport and biology. In 1989 Jason began his university education at Carnegie College (renamed Leeds Metropolitan University) and graduated with a B.A.(Hons) degree in human movement studies in 1992. Following this he worked in sales for Black and Decker UK for two years before deciding to leave England to pursue a Masters degree in America. Jason completed his M.Ed. at Western Carolina University (NC) in 1995 and progressed to Louisiana State University (Baton Rouge) for doctoral studies. Five years later he graduated and is currently working as a Research Associate in the Department of Medicine, Division of Cardiology at Duke University Medical Center, Durham, NC.