THE FLOW-MEDIATED DILATION RESPONSE TO ACUTE EXERCISE IN OVERWEIGHT MEN

Ryan A. Harris

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Kinesiology,
Indiana University
May 2007

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Doctoral Committee

Janet P Wallace, Ph.D.

David M Koceja, Ph.D.

Kieren Mather, M.D.

Timothy D Mickleborough, Ph.D.

Date of Oral Examination
(April 23, 2007)

As far as I can remember, I have always been a goal oriented person. This stage of my life is dedicated to achieving the goal I set to become a researcher, a philosopher, someone who can potentially make an impact. Goals are not always easy, nor are they achieved merely by the person who sets them. In order to achieve the goal of becoming a PhD, I (we) had to relocate to Indiana from sunny California. This transition was not easy, and there are many people who befriended me along the way.

First of all, I would like to thank Janet Wallace; my doctoral advisor, dissertation chair, and colleague. She has put forth a great commitment to help me achieve this goal. I would also like to express gratitude to Dave Koceja, Kieren Mather, Timothy Mickleborough, and Henry Prange for serving on my course prescription/research committee(s), sharing their wisdom with me, and giving me guidance along the way. To all my friends and colleagues who have played a role in helping me reach my goals at IU, you know who you are... THANK YOU! To my family who has supported me throughout my life and especially 2500 miles away here in Indiana, thank you for your love and support

Last but definitely not least, special thanks and all my love go out to my wife Staci, she was the one who kept me motivated throughout our four years in Bloomington; "When you finish, you can get a real job and we can move back to California!" Thank you and I LOVE YOU!

Ryan A. Harris

THE FLOW-MEDIATED DILATION RESPONSE TO ACUTE EXERCISE IN OVERWEIGHT MEN

INTRODUCTION Inflammation has been found to play a role in the etiology of coronary heart disease as well as induce endothelial dysfunction. Brachial artery flow-mediated dilation (FMD) is a measure of nitric oxide dependent vasodilation and represents a non-invasive measurement of endothelial function. The aim of the present study was to 1) investigate the interaction of inflammatory biomarkers on the FMD response to acute exercise in overweight men and 2) determine if FMD following moderate intensity acute exercise is reproducible.

METHODS Sixteen overweight men ages 46-68 years were classified as either being active or inactive. Subjects performed three acute exercise treatments (25%, 50%, and 75% VO_2peak), separated by at least two days apart. The 50% intensity was repeated in 9 subjects to investigate FMD reproducibility in response to acute exercise. Following the initial insertion of a venous catheter, brachial artery Flow-Mediated Dilation (FMD) and subsequent blood samples were taken pre exercise and every hour for three hours thereafter.

RESULTS The active group displayed a 24% increase ($p=.034$) in FMD following acute exercise compared to a 32% decrease ($p=.010$) in FMD observed in the inactive group. Both groups exhibited an elevated concentration of IL-6 following moderate (50% VO_2) and high (75% VO_2) intensity acute exercise ($p<.001$ and $p<.001$, respectively), whereas no change ($p=.669$) in IL-6 following low intensity (25% VO_2) exercise in either group was observed. No differences in TNF-α were observed between

groups (p=.433) or in response to acute exercise (p=.584). A significant FMD correlation (r = 0.531; p=.008) following exercise between trial 1 and trial 2 was found.

CONCLUSION FMD following exercise appears to be as reproducible as resting controlled conditions. In addition, the FMD response to acute exercise is enhanced in overweight active men when compared to their inactive counterparts; however, inflammation did not provide insight into the physiological mechanisms associated with the improvement of FMD.

This research was supported by the Gatorade Sport Science Institute and Indiana University HPER Research Grant-in-Aids.

TABLE OF CONTENTS

MANUSCRIPT

REPRODUCIBILITY OF THE FLOW-MEDIATED DILATION RESPONSE TO ACUTE EXERCISE IN OVERWEIGHT MEN

Running Title: Reproducibility of flow-mediated dilation

ABSTRACT

Flow-mediated dilation (FMD) has been established as a reliable non-invasive measurement of endothelial function. The reproducibility of FMD under resting conditions has previously been reported; however, the reproducibility of FMD in response to exercise remains to be investigated. On two separate days, we determined if flow-mediated dilation is reproducible in response to acute exercise in nine overweight men. Following pre-exercise FMD measurements, subjects were asked to walk on a treadmill for 45 minutes at 50% of their VO_2peak. Subsequently, FMD was measured immediately, and every hour for three hours thereafter. Reproducibility of FMD following exercise was assessed utilizing: 1) a two-way ANOVA, 2) Intra-class correlation coefficients (ICC), 3) Pearson correlations (r), 4) coefficient of variation (CV %), and coefficient of variation prime (CV') for FMD at each time period. Four acceptable reproducibility assessments were required to confirm FMD reproducibility in response to acute exercise. No differences ($F_{1,8}$ = .01; p=.942) in FMD were observed between trials collapsing for time. All the ICC_{FMD} fell within the reproducible criterion set and are as follows: pre-exercise 0.602, immediately post 0.840, 1 hr post 0.632, 2 hr post 0.724, and 3 hr post 0.631. The correlation and the average CV% for FMD between trials was 0.579 and 25.2%, respectively. The FMD response to an acute bout of moderate treadmill exercise appears to be as reproducible as FMD measured during resting conditions. The findings of the present study support the use of FMD as an outcome variable in response to acute exercise.

Key words: Intra-class correlation coefficient, Coefficient of variation, Endothelial-dependent vasodilation, Brachial artery, Physical activity

INTRODUCTION

Atherosclerotic cardiovascular disease remains the leasing cause of morbidity and mortality in the United States. Atherosclerosis is a progressive inflammatory disease characterized by the accumulation of lipids and fibrous elements in medium to large arteries (Lusis 2000) and has been suggested to be the result of endothelial dysfunction (Munro and Cotran 1988; Ross 1986). Endothelial cells participate in multiple essential functions to maintain vascular homeostasis and health, including regulation of vascular smooth muscle tone, control of thrombosis, inhibition of leukocyte and platelet adhesion, and promotion of intra-arterial permeability (Celermajer 1997; Rubanyi 1993; Vane et al. 1990), all of which contribute to atherosclerotic cardiovascular disease. Moreover, many clinical populations (Edwards et al. 2004; Guerci et al. 2001; Maiorana et al. 2003) present with endothelial dysfunction.

Flow-mediated dilation (FMD) is a non-invasive ultrasonic measurement of endothelial function (Uehata et al. 1997) which has been shown to correlate with invasive testing of coronary artery endothelial function (Anderson et al. 1995; Takase et al. 1998). The occlusion-induced production of reactive hyperemia to promote endothelial nitric oxide dependent vasodilation (ie. flow-mediated) evaluates the shear stress mechanism of NO production. FMD has been most commonly studied in the brachial artery (Smith et al. 2000) and is widely used and accepted as a marker of endothelial function.

For decades, the use of diet and exercise has been indicated as non-pharmacological interventions in the treatment and prevention of disease. Recently exercise has been found to preserve and/or reverse endothelial (dys)function (Edwards et al. 2004; Gokce et al. 2002; Hambrecht et al. 2003). There is substantial evidence suggesting an

improvement in FMD following exercise training in healthy and clinical populations (Gokce et al. 2002; Kasikcioglu et al. 2005) with the subsequent reduction of cardiovascular disease risk; however, the physiological mechanisms associated with the improvement in endothelial function are not fully understood (Brunner et al. 2005). Furthermore, 45 minutes of moderate intensity (acute) exercise has been shown to improve FMD (Harvey et al. 2005) and other measures of endothelial function (Bode-Boger et al. 1994; Camsari et al. 2003; Gill et al. 2004; Kingwell et al. 1997). The advantage of utilizing an acute paradigm allows for the investigation into possible physiological response mechanisms and efficient manipulation of exercise variables (i.e. intensity, duration, volume, etc.) both to detect changes in the outcome variable of interest, in this case FMD.

Although investigations are focusing on the benefit of exercise to improve endothelial function in various clinical populations and conditions, the reproducibility of FMD following exercise remains to be investigated. In view of the fact that exercise elicits many physiological changes (i.e. heart rate, blood pressure, sympathetic activity, etc.) that may influence the measurement of FMD, it seems imperative that the reproducibility of FMD following such stimulus is investigated. The purpose of the present study was to determine if flow-mediated dilation is reproducible in response to acute exercise in a population that is susceptible to endothelial dysfunction. Overweight men were recruited to represent a population susceptible to endothelial dysfunction; a cohort typically targeted in exercise intervention. It was hypothesized that the FMD response to a bout of moderate intensity exercise would be similar on two different days.

METHODS

Experimental Design

On two separate occasions, participants reported to the Indiana University Clinical Exercise Physiology Laboratory at 0730 hrs following an overnight fast (Corretti et al. 2002). The two trials were separated by at least 2 days to control for any training effects. Following pre-exercise FMD measurements, subjects were asked to walk on a motor driven treadmill for 45 minutes at 50% of their exercise capacity (VO_2peak). For each trial, FMD was assessed pre-exercise, immediately following exercise, and every hour for three hours thereafter.

Subjects

Nine overweight men aged 46 to 66 years old participated in this investigation. All subjects had prior physician approval to participate in this study. Subjects were excluded if they 1) had any known cardiovascular, pulmonary or metabolic diseases, 2) had orthopedic problems that would limit their exercise tolerance, and 3) were on any medications that influence arterial compliance. Following the guidelines for the ultrasonic assessment of endothelial dependent FMD of the brachial artery (Corretti et al. 2002), subjects were asked to abstain from physical activity, tobacco products, and vitamin supplementation for 12 hours prior to each trial day. All procedures were approved by the Indiana University Committee for the Protection of Human Subjects. Written informed consent was obtained from each subject prior to participation in the study.

Brachial artery Flow-Mediated Dilation

Endothelial function was measured via brachial artery flow-mediated dilation (FMD). FMD was assessed pre-exercise, immediately, and 1, 2, and 3 hours following the cessation of exercise. For each measurement (excluding the immediate), subjects were

instructed to lie supine for 20 minutes to establish a hemodynamic steady state in a climate controlled room (22°-24°C). The brachial artery was imaged longitudinally by a 2D high resolution Sonoace Pico ultrasound system (Universal Medical Systems, Bedford Hills, NY, USA) using a 7.0 MHz linear transducer, placed 2-10 cm above the antecubital fossa. Once a clear image was obtained, the transducer was placed in a stabilized holder and the position marked to ensure the same placement for each FMD measurement. Following 20 minutes of acclimation, the average diameter and blood velocity for 10 cardiac cycles were recorded and analyzed for baseline values. A 5 cm forearm occlusion cuff (D.E. Hokanson, Bellevue, WA, USA) was placed around the right forearm and rapidly inflated using compressed air (E-20 rapid cuff inflator, D.E. Hokanson, Bellevue, WA, USA) to induce occlusion and subsequent reactive hyperemia of the brachial artery. Forearm occlusion was maintained for 5 minutes at 250 mm Hg. Doppler measurements of peak hyperemic blood velocity were made during the first 10 seconds following rapid cuff release at an isonation angle of 70^{o} (Harris et al. 2006; Wright et al. 2006) before switching back to continuous 2D ultrasound imaging for the remainder of the 2 minute collection period. EKG gaiting was utilized to capture end-diastolic arterial diameters, triggered by the QRS complex, which were analyzed using the Vascular Analysis Integrative System (Medical Imaging Applications, Coralville, Iowa, USA). The highest 10 second averaged interval throughout the 2 minute collection period represented the peak hyperemic diameter. Hyperemic velocity and baseline diameter were converted to hyperemic local shear stress (HLSS) using the following equation (Mitchell et al. 2004): $HLSS = 8 \times \mu \times V_H/D_{BL}$, where μ is blood viscosity, assumed to be 0.035 dyne x s/cm^2, V_H is peak hyperemic velocity, and D_{BL} is baseline

diameter. FMD is expressed as a percent increase in diameter from baseline and calculated as: FMD = (peak hyperemic diameter – baseline diameter)/ baseline diameter. All image acquisitions were performed by the same investigator. In addition, all measurement analyses were performed by the same investigator who was blinded to the trial. Intra-observer reliability yielded an ICC of 0.987 with a variation of 2.2%.

Treadmill Exercise

Prior to the start of the investigation each subject performed a maximal treadmill exercise test to obtain their peak oxygen consumption (VO_{2peak}). VO_{2peak} was then used to calculate the moderate intensity exercise (50% of VO_{2peak}) to be performed for each trial. Following pre-exercise FMD measurements, subjects were instructed to walk on a motor driven treadmill at 50% VO_{2peak} for 45 minutes. Sensor Medics 2900 metabolic cart (Sensor Medics Corporation, Yorba Linda, CA, USA) was used to collect expired gases between the 5^{th} and 10^{th} minute to confirm the appropriate exercise intensity. The work rate was adjusted if the VO_2 was not within ± 5% of the target exercise intensity. Expired gases were then measured again between the 10^{th} and 15^{th} minute to confirm the new exercise intensity. Heart rate (EKG lead II), blood pressure (auscultation), and rating of perceived exertion (RPE; Borg Scale 6-20) were recorded every 5 minutes to ensure similar exercise induced stimuli between trials. For trial 2, the same speed and grade as the previous trial was utilized; however actual VO_2 was not measured.

Assessment of Reproducibility

Reproducibility of brachial artery FMD following exercise was assessed utilizing an ANOVA, Intra-class correlation coefficients (ICC), Pearson correlation coefficients (r), and coefficients of variation (CV) for: FMD, baseline diameter, peak hyperemic diameter, baseline blood velocity, peak hyperemic blood velocity, and hyperemic local

shear stress at each time period between trials. In addition, to determine the reproducibility of FMD in response to acute exercise, area under the curve (AUC) in arbitrary units (au) and a Bland-Altman (Bland and Altman 1986) plot were utilized. AUC for each trial was calculated by summing the area of successive trapezoids in relation to the pre-exercise values and assessed by ICC. The ICC, r, and CV was reported for the mean FMD at each time period between trials. Since FMD is expressed as a percent and is very sensitive to changes in baseline diameter, coefficients of variation prime (CV') has also been reported as a measurement of FMD reproducibility (Herrington et al. 2001) and utilized in our assessment. CV' was calculated and reported for the same variables as CV. ICC values of <0.40, 0.40-0.75, and >0.75 represent poor, fair to good, and excellent agreements, respectively (Landis and Koch 1977). A non-significant F-ratio, Intra-class correlation coefficients (ICC) and Pearson correlation coefficients greater than fair-to-good (Landis and Koch 1977), and CV's less than 35% were deemed acceptable. Four acceptable assessments were required to confirm reproducibility.

Statistical Analysis

Descriptive statistics were used to describe the sample characteristics. A two-way (trial x time) repeated measures ANOVA was performed to identify differences in: FMD, baseline diameters, peak hyperemic diameters, baseline blood velocity, peak hyperemic blood velocity, and hyperemic local shear stress. ICC, Pearson correlation coefficients and CV between trials were determined for FMD, baseline diameter, peak hyperemic diameter, baseline blood flow, peak hyperemic blood flow, and hyperemic local shear stress at each time period. The ICC for the FMD AUC between trials was also determined. A Bland-Altman plot was used to assess the agreement between trial 1 and

trial 2. CV and CV' were calculated for each subject from the mean and SD of both trials as follows: CV(%) = ((SD/mean) x 100) and CV' = ((100 x SD)/(mean + 100) (Herrington et al. 2001). All data are reported as mean ± SEM. Significance was set at $p<.05$.

RESULTS

Subject characteristics are presented in Table 1. Recorded exercise outcome variables were similar ($p>.05$) between trials: heart rate (110.11±5.4 vs. 107.1±5.5), systolic blood pressure (137.5±6.0 vs. 133.4±3.0), diastolic blood pressure (71.3±2.5 vs. 71.4±22.8), and RPE (11.54±0.53 vs. 11.48±0.55).

The AUC for the FMD response to exercise for each trial was -8.4±3.8 (au) and -4.8±2.1 (au). The ICC_{AUC} for the FMD response to exercise was 0.414. Figure 1 illustrates the FMD(%) response to a single bout of moderate intensity exercise between trials. The ANOVA indicated no differences ($F_{1,8} = .01$; $p=.942$) in FMD(%) between trial 1 and trial 2 collapsing for all time periods investigated. There were also no differences in baseline diameter ($F_{1,8} = .39$; $p=.549$), peak hyperemic diameter ($F_{1,8} = .94$; $p=.360$), baseline blood flow ($F_{1,8} = .42$; $p=.535$), peak hyperemic blood flow ($F_{1,8} = .64$; $p=.448$), and hyperemic local shear stress ($F_{1,8} = .31$; $p=594$) between trial 1 and trial 2 among all time periods investigated. The absolute difference in FMD(%) between the two trials was as follows: pre-exercise 0.79±1.00%, immediately post exercise 0.36±0.74%, 1 hr post exercise 0.51±0.72%, 2 hr post exercise 0.22±1.15%, and 3 hr post exercise 0.32±0.71%.

The ICC's and Pearson correlations for all variables at each individual time period are presented in Table 2. The mean ICC_{FMD} and Pearson correlation for each time period

between trials was 0.911; p=.019 and 0.958; p=.01, respectively. Figure 2 illustrates the significant correlation (r = 0.579; p<.001) between trial 1 and trial 2 utilizing all FMD(%) measurements. The Bland-Altman plot is illustrated in figure 3. Table 3 displays the CV and CV' for all investigated variables among the three hour time course studied. In summary, acceptable criteria was met for all assessments of reproducibility.

DISCUSSION

The present study investigated the reproducibility of FMD over a three hour time period in a population susceptible to endothelial dysfunction. The ANOVA detected no differences in FMD between trials (Figure 1), ICC and correlations were all $\geq$ 0.40 (Table 2), the average CV was well under the 35% criteria set (Table 3), and the Bland-Altman plot suggests an agreement between trial 1 and trial 2. These data support our hypothesis that the FMD response to moderate intensity acute exercise is reproducible. In addition, these findings confirm the use of an acute exercise paradigm when studying the favorable effects of exercise.

Roughly 60% of the United States population is overweight. Overweight men were recruited for this study to 1) represent a population susceptible to endothelial dysfunction and 2) elicit a response to the stimuli (exercise). Studies have shown an improvement in endothelial function in populations susceptible to endothelial dysfunction following acute exercise (Gaenzer et al. 2001; Harvey et al. 2005), whereas no change was observed in their *control* counterparts. Although the pre-exercise FMD did not appear compromised in our subjects, the acute exercise stimulus elicited a response in FMD that was consistently observed for both trials.

Investigations have been conducted to evaluate the stability of brachial artery FMD under controlled resting conditions, and report it to be reproducible (Hijmering et al. 2001; Welsch et al. 2002; West et al. 2004). In contrast, some researchers have found the measurement of FMD to be 'invalid' when observing FMD in healthy volunteers on multiple occasions mainly because of physiological factors (Hijmering et al. 2001) (ie. time of day and dietary intake) and reading variation (De Roos et al. 2003). By following the guidelines (Corretti et al. 2002), the confounding physiological factors suggested by Hijmering (2001) did not influence the robustness of our findings.

Exercise introduces physiological changes (i.e. sympathetic and vascular) which may influence the reproducibility of FMD. It has long been known that there are protective cardiovascular benefits associated with exercise. Most importantly, exercise plays a multifaceted beneficial role in the improvement of endothelial function as well as the reduction in cardiovascular disease risk. The intensity and duration of exercise utilized in the present investigation complies with the current exercise recommendation for prevention and treatment of disease (ACSM 2005). Moreover, the present study found similar responses to exercise in heart rate, blood pressure, and the subjects rating of perceived exertion between trials. FMD AUC was utilized to represent the global response to exercise. The AUC is a way to normalize the FMD response to pre-exercise values. Our results depict an acceptable ICC for the FMD AUC suggesting that each subject's response to exercise was similar on two separate days, which has never been reported to our knowledge.

There is no single ideal criterion used in practice to assess the reproducibility of brachial artery FMD (Corretti et al. 2002). Within any measurement of conduit artery

endothelial function, physiological factors may introduce a wide array of confounding variables. In an attempt to establish consistency and standardization when measuring brachial artery FMD, Corretti and colleagues (2002) published guidelines for the ultrasonic assessment of brachial artery endothelial function. These guidelines suggest performing correlation coefficients (r), mean differences, and coefficients of variations (CV) as a complete approach to report reproducibility of FMD. The present study incorporated multiple indices of reproducibility, including all three suggested by the guidelines.

Furthermore, there is no *concrete* threshold when observing the reproducibility of the FMD measurement. For example, in biological analysis, a CV $\leq$ 20% is traditionally accepted to define reproducibility of multiple assayed samples. Given that FMD is expressed as a percent increase, it is very sensitive to a small change in arterial diameter. It is unclear what the CV_{FMD} reported in literature represents. It may represent the CV of the baseline diameters, post hyperemic diameters, the standard deviation, or perhaps even the magnitude of change (De Roos et al. 2003). Due to the sensitive nature of the FMD measurement, the reported CV in literature have been above the acceptable CV for biochemical analysis; however, based on the guidelines of ultrasound assessment, a mean difference in FMD over time that is within 2-3% is acceptable (Corretti et al. 2002). In an average brachial artery FMD of 10%, this would translate to an FMD reproducibility criterion for CV's between 20-35%. West and colleagues (2004) reported a CV_{FMD} = 29.7% for individuals with Type 2 diabetes and stated their findings were within the low range of published values for CV_{FMD} of healthy individuals. The average CV_{FMD} reported in the present study falls below that reported by West and colleagues (2004) and is well

within the lower end of the calculated acceptable range suggested by the guidelines; albeit introducing *exercise* prior to the assessment of FMD reproducibility. In addition, the present study evaluates the reproducibility of FMD using several methods, of which all demonstrate FMD and the primary components (i.e. diameters, velocities, and shear stress) to be reproducible.

Day to day FMD variation under resting controlled conditions has previously been reported for healthy controls as well as in clinical populations (Kanani et al. 1999; Roos et al. 2002; Sorensen et al. 1995; Uehata et al. 1997). The CV_{FMD} for these investigations has ranged from 2-84% and has been assessed the same day or on different days. Variability in FMD is most likely a result of measurement error, biological variability, and differences in technique over repeated testing sessions (De Roos et al. 2003). Flow-mediated dilation variability may also be attributed to the uncontrolled nature of the evoked shear stress stimulus (Pyke et al. 2004), one of the most important stimulus for vasodilation. Pyke and colleagues (2004) demonstrate a reduction in FMD variation (CV_{FMD} = 36.16% vs. 51.8%, respectively) utilizing a controlled hyperemia test compared to a reactive hyperemia test. In the present study, the ICC_{HLSS} shows evidence of an excellent agreement between trials, suggesting similar stimuli on both days and validity of our FMD measurements. In addition, the ICC's for both baseline and peak hyperemic diameter between trials exhibit an excellent agreement which is consistent with literature (De Roos et al. 2003).

The sample size needed to assess reproducibility of FMD in recent published literature has ranged from as few as three subjects (Kanani et al. 1999) to as many as 127 subjects (Herrington et al. 2001). In addition, the number of FMD measurements has ranged from

2-6 in a single day period (De Roos et al. 2003) up to 2-5 one month apart (Uehata et al. 1997). The present study investigated nine subjects following exercise on two separate days which fall within the controlled FMD reproducibility power criteria reported in the literature (Hashimoto et al. 1995; Kanani et al. 1999; Lundman et al. 1997; Uehata et al. 1997).

Conclusions. Although FMD has been utilized as a primary non-invasive measurement of endothelial dysfunction following acute and chronic exercise, the present study is the first of its kind to evaluate the reproducibility of FMD following (acute) exercise. The findings of the present investigation support the hypothesis that FMD in response to acute exercise is reproducible. In addition, the inclusion of FMD reproducibility using AUC is a novel aspect of the present investigation. Our findings support the reproducibility of FMD as an outcome variable in response to acute exercise in subjects susceptible to endothelial dysfunction.

ACKNOWLEDGEMENTS

We want to thank all the subjects who dedicated their time and effort in making this investigation possible. This study was supported in part by the Gatorade Sport Science Institute research grant-in-aid and the Indiana University Health, Physical Education, and Recreation research grant-in-aid.

REFERENCES

ACSM. *ACSM's guidelines for exercise testing and prescription.* New York: Lippincott Williams and Wilkins, 2005, 368.

Anderson TJ, Uehata A, and Gerhard MD. Close relationship of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26(1235-1241.

Bland J and Altman D. Statistical methods for assessing aggreement between two methods of clinical measurement. Lancet 1986;i(307-310.

Bode-Boger SM, Boger RH, Schroder EP, and Frolich JC. Exercise increases systemic nitric oxide production in men. J Cardiovasc Risk 1994;1(2):173-178.

Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, Kiowski W, Luscher TF, Mancia G, Natali A, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Spieker LE, Taddei S, and Webb DJ. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the working group of endothelins and endothelial factors of the european society of hypertension. J Hypertens 2005;23(233-246.

Camsari A, Pekdemir H, Cicek D, Polat G, Akkus MN, Doven O, Cin G, Katircibasi T, and Parmaksiz T. Endothelin-1 and nitric oxide concentrations and their response to exercise in patients with slow coronary flow. circ J 2003;678(1022-1028.

Celermajer D. Endothelial dysfunction: does it matter? is it reversible? Journal of American College of Cardiology 1997;30(2):325-333.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gehard-Herman M, Herrington D, Vallance P, Vita J, and

Vogel R. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery. J Am Coll Cardiol 2002;39(2):257-265.

De Roos NM, Bots ML, Schouten EG, and Katan MB. Within-subject variability of flow-mediated dilation of the brachial artery in healthy men and women: Implications for experimental studies. Ultrasound in Med. and Biol. 2003;29(3):401-406.

Edwards DG, Schofield RS, Lennon SL, Pierce GL, Nichols WW, and Braith RW. Effect of exercise training on endothelial function in men with coronary artery disease. Am J Cardiol 2004;93(5):617-620.

Gaenzer H, Neumayr G, Marschang P, Sturm W, Kirchmair R, and Patsch JR. Flow mediated vasodilation of the femoral and brachial artery induced by exercise in healthy non smoking and smoking men. J Am Coll Cardiol 2001;38(1313-1319.

Gill JMR, Al-Mamari A, Ferrell WR, Cleland SJ, Packard CJ, Sattar N, Petrie JR, and Caslake MJ. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. J Am Coll Cardiol 2004;44(12):2375-2382.

Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keany JF, and Balady GJ. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. Am J Cardiol 2002;90(2):124-127.

Guerci B, Kearney-Schwartz A, Bohme P, Zannad F, and Drouin P. Endothelial dysfunction and type 2 diabetes. Diabetes Metab 2001;27(425-434).

Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, and Schuler G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phyosphorylation of endothelial nitric oxide synthase. Circulation 2003;107(3152-3158.

Harris RA, Padilla J, and Wallace JP. Variability of Flow-mediated Dilation Measurements with Repetitive Reactive Hyperemia. Vasc Med 2006;11(1):1-6.

Harvey PJ, Morris BL, Kubo T, Picton PE, Su WS, Notarius CF, and Floras JS. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. Hypertension 2005;23(285-292.

Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, and Ouchi Y. Modulation of Endothelium-Dependent Flow-Mediated Dilatation of the Brachial Artery by Sex and Menstrual Cycle. Circulation 1995;92(12):3431-3435.

Herrington DM, Fan L, Drum M, Riley WA, Pusser BE, Crouse JR, and Burke GL. Brachial flow mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. J Cardiovasc Risk 2001;8(5):319-328.

Hijmering ML, Stroes ESG, Pasterkamp G, Sierevogel M, Banga JD, and Rabelink TJ. Variability of flow-mediated dilation: consequences for clinical application. Atherosclerosis 2001;157(369-373.

Kanani PM, Sinkey CA, Browning RL, Allaman M, Knapp HR, and Haynes WG. Role of Oxidant Stress in Endothelial Dysfunction Produced by Experimental Hyperhomocyst(e)inemia in Humans. Circulation 1999;100(11):1161-1168.

Kasikcioglu E, Oflaz H, Kasikcioglu HA, Kayserilioglu A, Umman S, and Meric M. Endothelial flow mediated dilation and exercise capacity in highly trained endurance athletes. Tohoku J of Exp Med 2005;205(1):45-51.

Kingwell BA, Berry KL, Cameron JD, Jennings GL, and Dart AM. Arterterial compliance increases after moderate-intensity cycling. Am J Physiol 1997;273(H2186-H2191.

Landis J and Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(159-174.

Lundman P, Eriksson M, Schenck-Gustafsson K, Karpe F, and Tornvall P. Transient triglyceridemia decreases vascular reactivity in young, healthy men without risk factors for coronary heart disease. Circulation 1997;96(10):3266-3268.

Lusis A. Atherosclerosis. Nature 2000;407(233-241.

Maiorana A, O'Droscoll F, Taylor R, and Green D. Exercise and the Nitric Oxide Vasodilator System. Sports Med 2003;33(14):1013-1035.

Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF, Keyes MJ, Levy D, Vasan RS, and Benjamin EJ. Local shear stress and brachial artery flow-mediated dilation. Hypertension 2004;44(134-139.

Munro JM and Cotran RS. The pathogenesis of atherosclerosis: atherogenesis and inflammation. Lab Invest 1988;58(249-261.

Pyke KE, Dwyer EM, and Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. J Appl Physiol 2004;97(499-508.

Roos Nd, Siebelink E, Bots M, Van TA, Schouten E, and Katan M. Trans monounsaturated fatty acids and saturated fatty acids have similar effects on postprandial flow-mediated dilation. Eur J Clin Nutr 2002;56(674-679.

Ross R. The pathogenesis of atherosclerosis-an update. N Engl J Med 1986;314(488-500.

Rubanyi GM. The role of endothelium in cardiovascular homeostasis and disease. Journal of Cardiovascular Pharmacology 1993;22(4):S1-S14.

Smith S, Greenland P, and Grundy S. Conference preceedings. Prevention conference V. Beyond secondary prevention: identifying the high-risk patients for primary prevention. Executive summary. Circulation 2000;101(111-116.

Sorensen KE, Celermajer D, and Spiegelhalter D. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reprocucibility. Bristish Heart Journal 1995;74(247-253.

Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, and Kurita A. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998;82(12):1535-1539.

Uehata A, Lieberman EH, Gerhard MD, Anderson TJ, Ganz P, Polak JF, Creager MA, and Yeung AC. Noninvasive assessment of endothelium-dependent flow-mediated dilation of the brachial artery. Vascular Medicine 1997;2(87-92.

Vane JR, Anggard EE, and Botting RM. Regulatory functions of the vascular enodothelium. The New England Journal of Medicine 1990;323(27-36.

Welsch MA, allen JD, and Geaghan JP. Stability of reproducibility of brachial artery flow-mediated dilation. Med Sci Sports Exerc 2002;34(6):960-965.

West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Krick AL, Boseska L, Ulbrecht J, and hinderliter AL. Biological correlates of day to day variation in flow mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. Diabetologia 2004;47(1625-1631.

Wright SA, O'Prey FM, Rea DJ, Plumb RD, Gamble AJ, Leahey WJ, Devine AB, McGivern RC, Johnston DG, Finch MB, Bell AL, and McVeigh GE. Microcirculatory Hemodynamics and Endothelial Dysfunction in Systemic Lupus Erythematosus. Arterioscler Thromb Vasc Biol 2006;26(10):2281-2287.

TABLE/FIGURE LEGEND

Table 1.
Subject Characteristics

Table 2.
Intra Class Correlation Coefficients and Pearson correlations for each time period

Table 3.
Comparison of variables for both trials among all time periods investigated

Figure 1.
The FMD response to acute exercise on two separate days.

Figure 2.
The relationship of FMD across time between trial 1 and trial 2.

Figure 3.
Agreement of trial 1 and trial 2 using the Bland-Altman plot

Table 1. Subject Characteristics

Variables	
Age, years	57.1±2.3
BMI, kg/m^2	29.2±0.9
Systolic blood pressure, mm Hg	114±3.0
Diastolic blood pressure, mm Hg	75±3.2
VO_2peak, ml/kg/min	33.8±1.4
Total cholesterol, mg/dL	198.7±15.0
HDL cholesterol, mg/dL	48.3±5.3
LDL cholesterol, mg/dL	119.8±13.3
Triglycerides, mg/dL	153±25.0
Fasting glucose, mg/dL	94±1.6
C-reactive protein, mg/L	3.9±1.9

Table 2. Intra Class Correlation Coefficients and Pearson correlations for each time period

Variable	Pre-Exercise	Immediately Post	1 Hr Post	2 Hr Post	3 Hr Post
Flow-mediated dilation	0.602 / 0.432	0.840 / 0.734	0.632 / 0.486	0.724 / 0.573	0.631 / 0.464
Baseline diameter	0.958 / 0.923	0.975 / 0.953	0.933 / 0.879	0.949 / 0.949	0.979 / 0.972
Peak hyperemic diameter	0.930 / 0.876	0.983 / 0.967	0.941 / 0.890	0.977 / 0.961	0.966 / 0.938
Baseline blood velocity	0.711 / 0.558	0.577 / 0.415	0.560 / 0.400	0.543 / 0.414	0.577 / 0.475
Peak hyperemic blood velocity	0.613 / 0.442	0.827 / 0.705	0.767 / 0.689	0.812 / 0.734	0.770 / 0.680
Hyperemic local shear stress	0.854 / 0.748	0.931 / 0.871	0.860 / 0.800	0.865 / 0.871	0.876 / 0.879

Values are presented as ICC / r. ICC's and r $\geq$0.400 were deemed acceptable.

Table 3. Comparison of variables for both trials among all time periods investigated

	Pre-Exercise		Immediately Post		1 Hr Post		2 Hr Post		3 Hr Post		**Average CV/CV' (%)**
Variable	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	
Flow-mediated dilation (%)	7.8±1.0	7.0±0.9	5.0±0.9	5.3±1.1	5.4±0.6	5.9±0.8	5.9±1.2	5.7±1.3	5.0±0.7	5.4±0.6	25.2/1.3
Baseline diameter (mm)	4.13±0.14	4.08±0.13	4.15±0.15	4.12±0.15	4.16±0.16	4.13±0.14	4.07±0.16	4.06±0.12	4.13±0.16	4.10±0.13	2.3/0.1
Peak hyperemic diameter (mm)	4.46±0.16	4.36±0.14	4.36±0.15	4.33±0.16	4.40±0.15	4.35±0.15	4.32±0.18	4.28±0.16	4.32±0.15	4.32±0.13	2.0/0.1
Baseline blood velocity (cm/s)	20.9±3.0	21.8±3.4	25.2±1.8	29.2±2.3	17.6±2.2	15.8±2.3	13.3±1.3	13.5±2.1	11.0±0.8	11.9±1.4	18.0/2.8
Peak hyperemic blood velocity (cm/s)	93.9±4.7	91.4±4.7	87.9±4.8	88.9±4.6	97.3±7.4	84.6±4.2	80.3±3.7	82.1±4.3	79.2±4.9	83.7±5.9	8.9/4.2
Hyperemic local shear stress (AU)	6.5±0.5	6.6±0.5	6.3±0.5	6.2±0.5	6.0±0.5	6.5±0.6	5.9±0.5	6.0±0.6	6.1±0.7	5.8±0.5	10.0/0.6

Values are mean± SEM. CV = ((SD/mean) x 100); CV' = ((100 x SD)/(mean + 100).

No significant ($p<.05$) differences were observed between trials.

Figure 1.

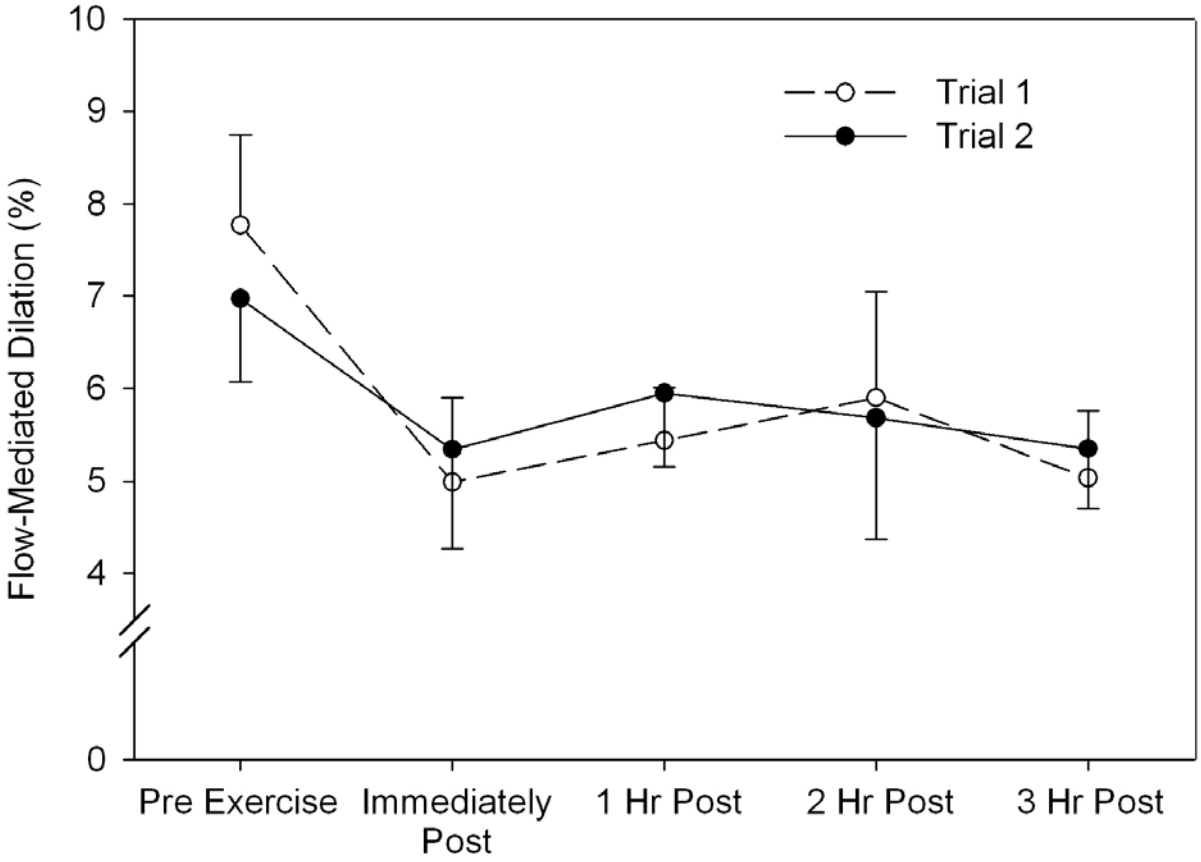

Flow-Mediated Dilation (%)
10
9
8
7
6
5
4
0
Trial 1
Trial 2
Pre Exercise
Immediately Post
1 Hr Post
2 Hr Post
3 Hr Post

Figure 2.

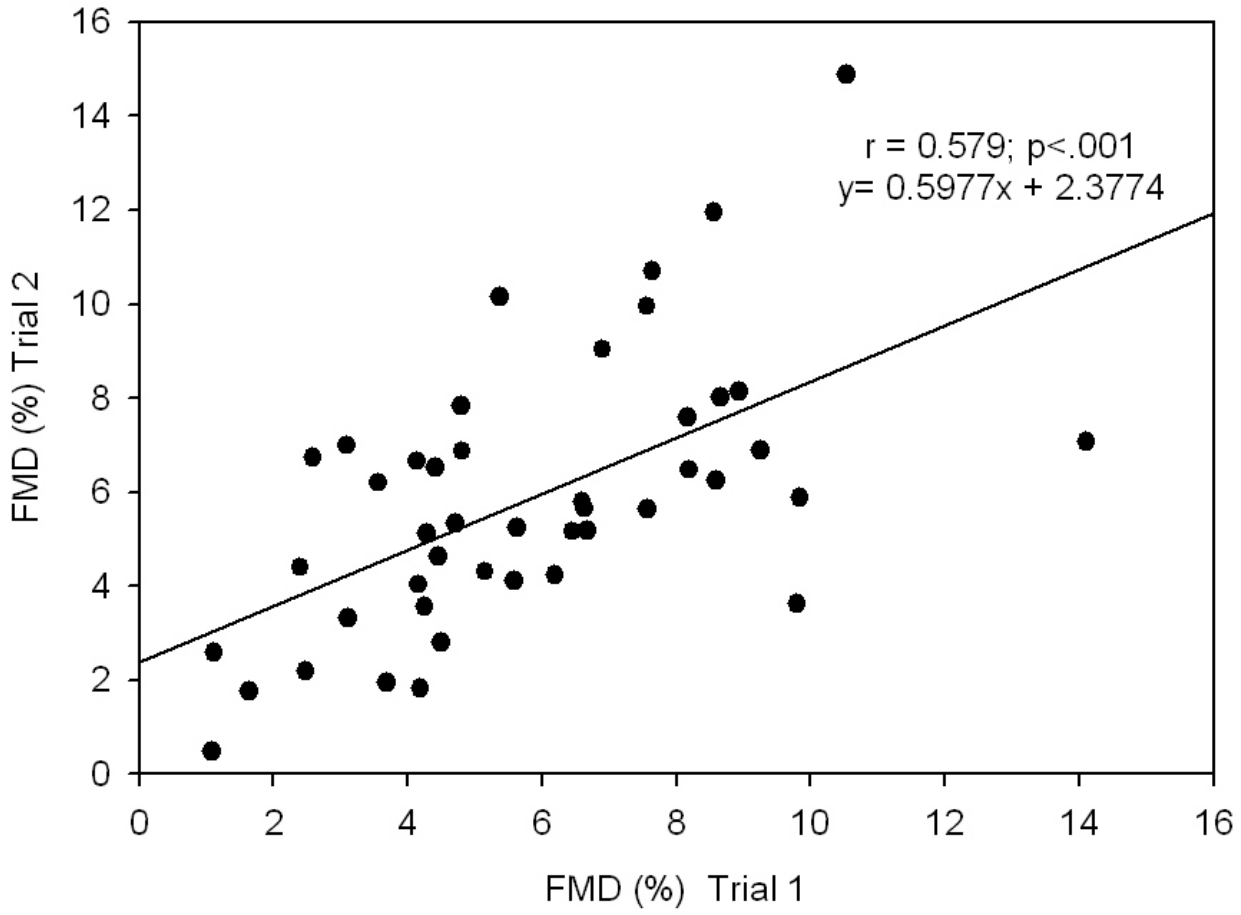
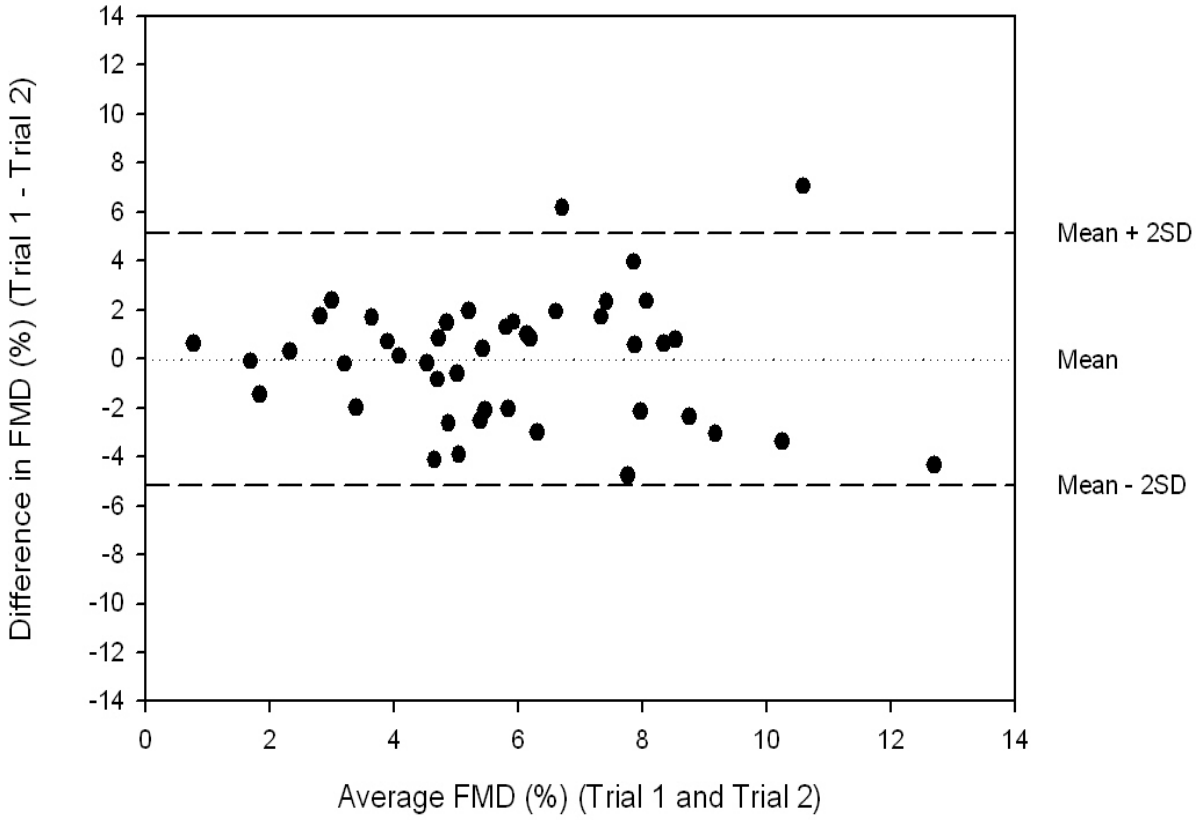

r = 0.579; p<.001
y= 0.5977x + 2.3774
FMD (%) Trial 2
FMD (%) Trial 1
0
2
4
6
8
10
12
14
16

Figure 3.


Difference in FMD (%) (Trial 1 - Trial 2)
Average FMD (%) (Trial 1 and Trial 2)
Mean + 2SD
Mean
Mean - 2SD
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
0
2
4
6
8
10
12
14

MANUSCRIPT

THE INTERACTION OF IL-6 AND TNF-α ON THE FMD RESPONSE TO ACUTE EXERCISE IN OVERWEIGHT ACTIVE AND INACTIVE MEN

Running Title: Acute Exercise and FMD in Overweight

ABSTRACT

Background – Inflammation has been found to play a role in the etiology of cardiovascular disease as well as provoke endothelial dysfunction. Inflammatory cytokines associated with endothelial function include IL-6 and TNF-α. IL-6 is exercise intensity dependent and has been shown to directly inhibit TNF-α expression. The aim of the present study was to investigate the interaction of IL-6 and TNF-α on endothelial function in response to acute exercise in overweight men exhibiting different physical activity profiles.

Methods and Results – In a randomized mixed factorial design 16 overweight men (8 active, VO_2peak = 34.2±1.7, BMI = 27.4±0.7 and 8 inactive, VO_2peak = 30.9±1.2, BMI = 29.3±1.0) performed three different intensity acute exercise treatments. Brachial artery Flow-Mediated Dilation (FMD) and subsequent blood samples were taken pre-exercise and 1 hr following the cessation of exercise. Although intensity did not influence the FMD response, a significant (P =.002) group x time interaction indicated the two groups responded differently. Elevated (P<.001) concentrations of IL-6 following moderate (50% VO_2) and high (75% VO_2) intensity acute exercise were observed in both groups; however, concentrations of TNF-α were unchanged in response to acute exercise (P =.584).

Conclusion – The FMD response to acute exercise is enhanced in *active* men who are overweight, whereas *inactive* men who are overweight exhibit an attenuated response. The interaction of IL-6 and TNF-α did not provide insight into the physiological mechanisms associated with the differential FMD response observed between groups.

Key Words: physical activity, endothelial function, inflammation, cardiovascular disease

INTRODUCTION

Obesity has emerged as a problematic epidemic in the United States and has recently been considered an independent risk factor for atherosclerotic cardiovascular disease.[1] Atherosclerosis is a progressive inflammatory disease characterized by the accumulation of lipids and fibrous elements in medium to large arteries [2] and is a manifestation of endothelial dysfunction.[3, 4] Brachial artery flow-mediated dilation (FMD) is a measure of nitric oxide dependent vasodilation and represents a non-invasive measurement of endothelial function. Unsurprisingly, individuals who are overweight or obese exhibit an increase in systemic inflammation[5] and are more likely to develop risk factors and co-morbidities of cardiovascular disease. There appears to be a positive relationship between overweight and all cause mortality in men[6]; however, overweight men who are active *may* have a lesser risk for heart disease than their sedentary counterparts.[7]

Inflammation has been found to play a role in the pathogenesis of coronary heart disease and provoke endothelial dysfunction.[8, 9] High sensitive C-reactive protein (CRP)[10], Tumor Necrosis Factor- Alpha (TNF-α)[11], and Interleukin-6 (IL-6)[12] are all bio-markers of systemic inflammation which have been associated with future atherosclerotic events. Recently, IL-6 has been proposed to provide anti-inflammatory effects[13] and accepts the role as a myokine,[14] a cytokine secreted from active skeletal muscle. The production of IL-6 is exercise intensity dependent[13] and has been shown to directly inhibit TNF-α expression in cardiac muscle[15] and skeletal muscle.[13] In a longitudinal study, Drenth and colleagues[16] found that exercise (training) increased IL-6, attenuated TNF-α, and reduced the acute inflammatory response. Consequently, the augmentation of FMD associated with acute exercise[17, 18] may be linked to the exercise induced IL-6 mediated TNF-α suppression; which has not been investigated. The physiological

mechanisms underlying the anti-atherogenic effects of the exercise associated improvements in endothelial function remain ambiguous.[19] The specific counteracting effects of IL-6 observed on TNF-α[13, 15] has led us to believe that the exercise induced increase in IL-6 may play a role in regulating the pro-inflammatory response and subsequent enhancement in FMD.

The aim of the present study was to investigate the interaction of IL-6 and TNF-α on the FMD response to acute exercise of different intensities in overweight men exhibiting different physical activity profiles. It was hypothesized that 1) the FMD response following acute exercise would be enhanced in overweight active men when compared to their inactive counterparts 2) baseline CRP and TNF-α would be elevated in the inactive men who are overweight compared to their active counterparts, 3) IL-6 would exhibit a positive exercise intensity dependent association, which would decrease the concentrations of TNF-α and provide a possible mechanism associated with the improvement in FMD.

METHODS

Experimental Design

In a randomized mixed factorial design subjects performed three different acute exercise treatments, separated by at least two days apart. Prior to each exercise investigation day, subjects were instructed to report to the Indiana University Clinical Exercise Physiology Laboratory at 0730 hrs having 1) fasted from the night before, 2) abstained from exercise for 24 hrs, and 3) abstained from caffeine and tobacco for 12 hrs. Following an initial left upper extremity venous catheter placement, brachial artery Flow-Mediated Dilation (FMD) and subsequent blood samples were taken pre-exercise and 1

hr following the cessation of exercise. Subjects were asked to remain in the laboratory and perform sedentary activities between measurements.

Subjects

Sixteen overweight men ages 46 to 68 years participated in this investigation. Subjects were classified into either an active (n=8) or inactive (n=8) group. Overweight was defined as having a body mass index (BMI) $\geq$ 25 kg/m^2. Physically active was defined following the Surgeon General's guidelines[20]; 30 minutes of moderate physical activity most days of the week. Subjects were excluded if they 1) had prior history of cardiovascular disease, pulmonary disease or diabetes, 2) could not exercise at 75% VO_2 peak for 45 minutes, 3) had a known allergy to heparin, 4) had orthopedic problems that would limit their exercise, or 5) were on any medications that influence vascular compliance. Subjects were asked to abstain from prophylactic aspirin and vitamin supplementation three days prior to the start of the study and throughout the investigation time period. All procedures were approved by Indiana University's Committee for the Protection of Human Subjects and the Institutional Biosafety Committee. Written informed consent was obtained from each subject prior to participation in the study.

Risk Stratification and Screening

Eligibility to participate in this investigation was determined by risk stratification. A risk stratification form detailing information of medical/family history of disease, height and weight, and current medications was completed by all subjects following initial informed consent. In addition, a fasting venous blood draw was performed to obtain CRP, total serum cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol.

Maximal Graded Exercise Test

The maximal graded exercise test was performed to measure the subjects maximal exercise capacity (VO_{2peak}) and obtain each subjects individual exercise treatment intensity. Briefly, subjects walked at a predetermined speed on a motor-driven treadmill with the grade increasing 2.5% every two minutes until volitional fatigue. Expired gases were collected into a mixing chamber through a unidirectional flow mouthpiece and analyzed using a Sensor Medics 2900 Metabolic Cart (Sensor Medics, Yorba Linda, CA). A maximal test was confirmed using the American College of Sports Medicine maximal exercise test criteria.

Flow-Mediated Dilation Acquisition/Analysis

Endothelial function was measured via brachial artery Flow-Mediated Dilation (FMD). FMD was measured pre-exercise and 1 hr following the cessation of exercise. For each FMD measurement, subjects were instructed to lie supine in a climate controlled room (22°-24°C) for 20 minutes to establish a hemodynamic steady state. The brachial artery was imaged longitudinally by a 2D high resolution Sonoace Pico ultrasound system (Universal Medical Systems, Bedford Hills, NY) using a 7.0 MHz linear transducer, placed 2-10 cm above the antecubital fossa. Once a clear image was obtained, the transducer was stabilized in a holder and the position was marked to ensure the same placement for each FMD measurement. The average diameter and blood velocity for 10 cardiac cycles was recorded and analyzed for baseline values. Following baseline measurements, A 5 x 84 cm forearm occlusion cuff (D.E. Hokanson, Bellevue, WA) was placed around the subjects' right forearm and rapidly inflated (E-20 rapid cuff inflator, D.E. Hokanson, Bellevue, WA) to induce occlusion for 5 minutes at 250 mm Hg and subsequent reactive hyperemia. Doppler measurements of peak hyperemic blood

velocity were made during the first 10 seconds following cuff release at an isonation angle of 70^{o} before switching back to continuous 2D ultrasound imaging for the remainder of the 2 minute collection period. EKG gaiting was utilized to capture end-diastolic arterial diameters, triggered by each QRS complex, which were analyzed using the Vascular Analysis Integrative System (Medical Imaging Applications, Coralville, Iowa). The highest 10 second averaged interval throughout the 2 minute post occlusion collection period represented the peak hyperemic diameter. Hyperemic velocity and baseline diameter were converted to hyperemic local shear stress (HLSS) using the following equation[21]: HLSS (arbitrary units, AU) = $8 \times \mu \times V_H/D_{BL}$, where μ is blood viscosity, assumed to be 0.035 dyne x s/cm^2, V_H is peak hyperemic velocity, and D_{BL} is baseline diameter. FMD is expressed as a percent increase in diameter from baseline and calculated as: FMD = (peak hyperemic diameter – baseline diameter)/ baseline diameter. All image acquisitions were performed by the same investigator. In addition, all measurement analyses were performed by the same investigator who was blinded to the exercise treatment.

Acute Exercise Treatments

Immediately following pre-exercise measurements, subjects' performed either a low (25% VO_{2peak}), moderate (50% VO_{2peak}), or high (75% VO_{2peak}) intensity treadmill walking session for 45-minutes. Each exercise treatment was separated by at least 2 days to eliminate any training effect. Oxygen uptake (VO_2) was measured via a Sensor Medics 2900 metabolic cart between the 5^{th} and 10^{th} minute of each exercise treatment to confirm the appropriate exercise intensity. The work rate was adjusted if the VO_2 was not within ± 5% of the target exercise intensity. Expired gases were then measured again between the 10^{th} and 15^{th} minute to confirm the new exercise intensity. Heart rate

(EKG), blood pressure (auscultation), and rating of perceived exertion (Borg Scale 6-20) were measured every 5 minutes throughout each exercise session.

Laboratory Procedures

All blood samples were collected from each subject and transferred into ethylenediaminetetraacetic acid (EDTA) tubes after an overnight fast (pre-exercise) and 1 hr following the cessation of exercise. All blood was centrifuged immediately at 1,000 x g for 15 minutes. The plasma supernatant was immediately transferred into microtubes and stored at -80°C until analyzed. Concentrations of TNF-α and IL-6 were measured using a high sensitive Enzyme-Linked Immunosorbent Assay (ELISA) kit according to manufacture specifications (R & D Systems, Minneapolis, MN). The mean detection limits of the kits are 0.106 pg/mL and 0.039 pg/mL for TNF-α (4^{th} generation) and IL-6 (3^{rd} generation), respectively. All samples were run in triplicate. Sample concentrations that fell above a coefficient of variation (CV) of 20% were re-run. The mean concentration of each sample was used in the statistical analyses. Concentrations of CRP, triglycerides, cholesterol and lipid sub-fractions (HDL and LDL) were obtained by standard laboratory procedures and only collected for pre-investigation characteristic values.

Statistical Analysis

Descriptive statistics and independent *t* tests were utilized to compare subject demographics and exercise induced physiological variables between groups. To determine the FMD, IL-6 and TNF-α response to acute exercise, comparisons were made using three-way (group x intensity x time) mixed factorial ANOVA's (SPSS Inc., Chicago, IL, USA: V 14.0). For any significant interactions, simple main effects were

employed. When indicated by a significant *F*-ratio, Dunnett's post hoc analysis was performed to identify differences. All data are expressed as mean ± standard error of the mean (SEM). Statistical significance was set at $P<0.05$.

RESULTS

Subject Characteristics

Sixteen subjects were recruited for this investigation and were placed into either an active (n=8) or inactive (n=8) group based on their physical activity profile. Table 1 summarizes the subject's characteristics. The active group reported more physically active days per week when compared to the inactive group (4.4±0.5 vs. 0.7±0.3; ($P<.001$), whereas all other physical characteristics were similar between the two groups.

HR, BP, RPE, and VO_2 in Response to Acute Exercise

Table 2 illustrates the heart rate (HR), blood pressure (BP), rating of perceived exertion (RPE), and oxygen consumption (VO_2) in response to acute exercise of different intensities. Oxygen consumption is presented as the absolute VO_2 obtained in response to acute exercise, as well as the VO_2 obtained expressed as a percentage of VO_2peak. No differences in exercise induced physiological variables were identified among acute exercise intensities between groups.

FMD in Response to Acute Exercise

Figure 1 illustrates the effect of exercise intensity on the FMD response 1hr following the cessation of exercise in overweight men who are active and inactive. For both groups, the FMD response was similar ($F_{2,28} = .02$; $P = .982$) independently of exercise intensity. Although intensity did not influence the FMD response, a significant ($F_{1,14} = 14.22$; $P = .002$) group x time interaction indicated the two groups responded differently.

Pre-exercise FMD was similar ($F_{1,14}$ = 0.21; *P* =.654) between groups; therefore, the FMD values were normalized to reflect the absolute response to exercise. Figure 2 illustrates the group x time interaction ($F_{1,14}$ = 14.22; *P* =.002)for post-exercise FMD normalized to pre-exercise values. Independent of exercise intensity, the active group displayed a 24% increase ($F_{1,14}$ = 5.49; *P* =.034) in FMD following acute exercise compared to a 32% decrease ($F_{1,14}$ = 8.95; *P* =.010) in the inactive group. Baseline arterial diameters, baseline and hyperemic blood velocities, and hyperemic local shear stress are presented in Table 3. For both groups, baseline diameters decreased (*P*=.03) following the low intensity acute exercise, no change (*P*=.725) was observed for the moderate intensity exercise; however, an increase (*P*=.002) following high intensity acute exercise was observed. In addition, baseline blood velocity was decreased (*P*=.019) 1 hr following exercise in both groups independent of exercise intensity. The active group displayed a decrease (*P*=.006) in hyperemic velocity following acute exercise independent of exercise intensity, whereas no change was observed in the inactive group. It is important to note that no differences in hyperemic local shear stress upon cuff release, a stimulus for vasodilation, were observed for either group throughout the analysis.

IL-6 in Response to Acute Exercise

Pre-exercise concentrations of IL-6 were similar ($F_{1,14}$ = 1.38; *P* =.259) between the active and inactive groups (1.3±0.3 pg/mL vs. 1.8±0.3 pg/mL, respectively). ANOVA indicated a significant ($F_{2,28}$ = 27.17; *P*<.001) intensity x time interaction for the IL-6 response to exercise. Figure 3 illustrates, for both groups, a significant elevation in plasma concentrations of IL-6 following moderate and high intensity acute exercise ($F_{1,28}$

= 27.32; P<.001 and $F_{1,28}$ = 99.61; P<.001, respectively), whereas no change ($F_{1,28}$ = 0.19; P =.669) in IL-6 was observed following low intensity exercise.

TNF-a in Response to Acute Exercise

No differences ($F_{1,14}$ = 0.31; P =.584) in pre-exercise concentrations between groups were observed. In addition, no change ($F_{1,14}$ = .00; P =.983) in plasma TNF-α in response to acute exercise was observed in either group. Figure 4 illustrates plasma concentrations of TNF-α in response to different intensities of acute exercise between groups.

DISCUSSION

The aim of the present study was to investigate the interaction of IL-6 and TNF-α on the FMD response to acute exercise of different intensities in overweight men exhibiting different physical activity profiles. Our findings support the main hypothesis that FMD in response to acute exercise would be enhanced in overweight active men when compared to their inactive counterparts; however, the pro- and anti- inflammatory state pre- as well as post- exercise may not explain the improvement in FMD observed.

Elevated BMI is associated with higher concentrations of CRP.[5] It was hypothesized that the state of global systemic inflammation would differ between overweight men who exhibited different physical activity profiles. Although the baseline characteristic of CRP does not exhibit a statistical difference, there appeared to be a trend towards separation between the two groups. In addition, contrary to our original hypothesis, there is no difference in the pre-exercise concentrations of TNF-α between groups. This similarity may be explained by a recent study by Van Guilder and colleagues[22] who found similar concentrations of TNF-α between normal weight and obese subjects; however, the obese

who presented with metabolic syndrome exhibited significantly elevated concentrations of TNF-α when compared to the normal weight subjects and the obese without metabolic syndrome. Further, the similar TNF-α response to acute exercise leads us to believe that the change in FMD observed in overweight men is independent of this plasma marker of inflammation.

TNF-α is postulated to be the first responder of the pro-inflammatory cytokine family, whereas IL-6 is typically the last responder.[16] The production of IL-6 during exercise is related to the intensity and duration of the exercise.[13] The findings of the present study strongly support this phenomenon. It is possible that the increase in IL-6 in response to moderate and high intensity exercise inhibited the TNF-α response, however, the response of IL-6 and TNF-α to acute exercise was similar in both groups, which does not explain the different response in endothelial function. Although no changes in plasma TNF-α were observed, evidence supports an increase in myocardial TNF-α expression immediately following an acute bout of moderate intensity exercise of similar duration.[23] It is important to note that a possible increase in TNF-α may have appeared prior to the 1 hr post-exercise time period evaluated in the present study; again, the similar cytokine concentrations pre- and post- exercise observed between the two groups would suggest the timing of the post-exercise measurement did not influence the difference in FMD observed. There are many benefits from habitual exercise that may elucidate cardioprotective effects and explain the different FMD response observed between the two groups.

There is substantial evidence suggesting that habitual exercise enhances FMD in healthy as well as clinical populations.[24, 25] Exercise training has been shown to augment blood flow and shear stress, which in turn increases NO production, leads to an increase

in NO bioavailability,[26] and enhances endothelial function.[27] A strong association exists between inflammation and training intensity[28, 29] which may have an impact on the FMD response to exercise. In the present study, both groups received the same relative exercise intensity (stimuli) which is evident by the similar response of physiological variables. Further, the present study investigated the FMD in response to acute exercise, there does not appear to be an effect of exercise intensity for either group. These findings are in contrast to Goto and colleagues[30] investigation who have reported an improvement in endothelial function following moderate intensity exercise training, whereas no change following low or high intensity training was discovered. The fact that there was a differential FMD response between groups; independent of exercise intensity, may suggest the use of a single exercise intensity when utilizing an acute exercise model. Evidence suggests that an acute bout of exercise can reduce triglycerides, reduce blood pressure, increase high density lipoprotein cholesterol, improve insulin sensitivity, and improve glucose homeostasis;[31] therefore, utilizing an acute exercise model to investigate the anti-atherogenic effects in endothelial function is supported. In general, the findings of the present investigation support the school of thought that being fit in overweight may reduce the hazards of obesity.[6, 32, 33] More specifically, habitual exercise may reduce vascular expression of NAD(P)H oxidase which results in decreased local reactive oxygen species generation (ROS),[34] increase antioxidant status,[35] and/or improve insulin sensitivity.[36]

The increase in oxygen uptake during acute exercise produces an increase in oxidative stress (OS),[37] which has been shown to impair endothelial function.[38] The acute exercise-induced OS may conceptually appear to contradict the enhancement in FMD observed following exercise.[17, 18] Evidence supports an exacerbated oxidative stress response to a

single bout of exercise in obese subjects;[37] however, active individuals have a greater resistance to acute exercise-induced oxidative stress.[35] The extent of oxidative damage is not only a factor of ROS generation, but also the capacity of antioxidant defense.[35] The resistance to acute exercise-induced ROS may be through the mechanism of (exercise) training induced enhanced antioxidant capacity.[35] Although, subjects were instructed to abstain from vitamin supplementation throughout this investigation, the dietary influence of antioxidants between the active and inactive overweight men is unknown and may have influenced the FMD response. The balance of pro- and anti- oxidant status may provide explanation to the endothelial function observed in response to acute exercise in the present study. In addition, the improvement in insulin sensitivity associated with habitual exercise in obesity may be related to changes in skeletal muscle fatty acid metabolism and the enhancement of post absorptive fat oxidation.[39] We can only speculate to the possible mechanism(s) involved in the different FMD responses observed in the present study. The physiological adaptations proposed may support the potential mechanism(s) associated with our findings; however, to our knowledge they have not been investigated in overweight or obesity following an oxidative challenge such as acute exercise.

In conclusion, the present study is the first of its kind to evaluate the FMD response to acute exercise of different intensities in overweight men. Our findings support the hypothesis that the FMD response to acute exercise is enhanced in overweight active men when compared to the attenuation observed in their inactive counterparts; however, the interaction of IL-6 and TNF-α does not support the disparity in FMD observed in response to acute exercise. In general, it appears that physical activity profile is an independent determinant of the FMD response to acute exercise observed between the

two groups. More specifically, habitual physical activity may result in an improvement in oxidative stress, antioxidant capacity, insulin resistance, and/or a combination of all three which may explain our results; however, future investigations of this kind in overweight and obesity are warranted.

ACKNOWLEDGEMENT

We would like to thank all the subjects who dedicated their time and effort in making this investigation possible. This research was supported in part by the Gatorade Sport Science Institute Research Grant-in-aid and the Indiana University Health, Physical Education, and Recreation Research Grant-in-Aid.

REFERENCES

1. Eckel RH, Krauss RM. American Heart Association Call to Action: Obesity as a Major Risk Factor for Coronary Heart Disease. *Circulation.* June 2, 1998 1998;97(21):2099-2100.
2. Lusis A. Atherosclerosis. *Nature.* 2000;407:233-241.
3. Ross R. The pathogenesis of atherosclerosis-an update. *N Engl J Med.* 1986;314:488-500.
4. Munro JM, Cotran RS. The pathogenesis of atherosclerosis: atherogenesis and inflammation. *Lab Invest.* 1988;58:249-261.
5. Visser. Elevated C-Reactive Protein Levels in Overweight and Obese Adults. *JAMA.* 1999;282(22):2131-2135.
6. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH. Physical fitness and all cause mortality. *JAMA.* 1989;262:2395-2401.
7. Lee CD, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *Am J Clin Nutr.* 1999;69:373-380.
8. Ross R. Atherosclerosis - An inflammatory disease. *N Engl J Med.* 1999;340(2):115-126.
9. Stenvinkel P. Endothelial dysfunction and inflammation-is there a link? *Nephrol Dial Transplant.* 2001;16:1968-1971.
10. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results

from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation.* 1999;99(2):237-242.

11. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation.* 2000;101(18):2149-2153.
12. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation.* 2000;101(15):1767-1772.
13. Pedersen BK, Steensberg A, Schjerling P. Muscle-derived interleukin-6: possible biological effects. *J Physiol.* 2001;536(2):329-337.
14. Febbraio MA, Pedersen BK. Contraction induced myokine production and release: Is skeletal muscle an endocrine organ. *Exerc Sport Sci Rev.* 2005;33(3):114-119.
15. Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK. IL-6 and TNF-a expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2002;283:E1272-E1278.
16. Drenth JP, Van Uum SH, Van Deuren M, Pesman GJ, Van der Ven-Jongekrijg J, Van der Meer JW. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production. *J Appl Physiol.* November 1, 1995 1995;79(5):1497-1503.
17. Benjamin E, Larson M, Keys MJ, Mitchell GF, Vasan RS, Keaney JF, Lehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: The framingham heart study. *Circulation.* 2004;109:613-619.

18. Harvey PJ, Morris BL, Kubo T, Picton PE, Su WS, Notarius CF, Floras JS. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *Hypertension.* 2005;23:285-292.

19. Higashi Y, Yoshizumi M. Exercise and endothelial function: Role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther.* 2004;102:87-96.

20. U.S.Department of Health and Human Services. Physical Activity and Health: A Report of the Surgeon General. In: Services USDoHaH, Prevention CfDCa, Promotion NCfCDPaH, eds. Atlanta, GA; 1996.

21. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF, Keyes MJ, Levy D, Vasan RS, Benjamin EJ. Local shear stress and brachial artery flow-mediated dilation. *Hypertension.* 2004;44:134-139.

22. VanGuilder G, Hoetzer G, Greiner J, Stauffer B, DeSouza C. Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity.* 2006;14(12):2127-2131.

23. Yamashita N, Kamiya K, Yamada K. Experience with a programmable valve shunt system. *Journal of Neurosurgery.* 1999;91(1):26-31.

24. Gokce N, Vita JA, Bader DS, Sherman DL, Hunter LM, Holbrook M, O'Malley C, Keany JF, Balady GJ. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol.* 2002;90(2):124-127.

25. Kasikcioglu E, Oflaz H, Kasikcioglu HA, Kayserilioglu A, Umman S, Meric M. Endothelial flow mediated dilation and exercise capacity in highly trained endurance athletes. *Tohoku J of Exp Med.* 2005;205(1):45-51.

26. Higashi Y, Oshima T, Ozono R. Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension.* 1997;30:252-258.

27. Wang J, Wolin MS, Hintze TH. Chronic exercise enhances endothelium-mediated dilation fo epicardial coronary artery in conscious dogs. *Circ Res.* 1993;73:829-838.

28. Forjaz CL, Cardoso CG, Rezk CC, Santaella DF, Tinucci T. Postexercise hypotension and hemodynamics: the role of exercise intensity. *J Sports Med and Phys Fit.* 2004;44(1):54-62.

29. Kemi OJ, Haram PM, Loennechen JP, Osnes J, Skomedal T, Wisloff U, Ellingsen O. Moderate vs high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovasc Res.* 2005;67:161-172.

30. Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, Kawamura M, Chayama K, Yoshizumi M, Nara I. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans. Role of endothelium-dependent nitric oxide and oxidative stress. *Circulation.* 2003;108:530-535.

31. Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L. The acute versus chronic response to exercise. *Med Sci Sports Exerc.* 2001;33(6):S438-S445.

32. DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, Seals DR. Regular aerobic exercise prevents and restores age-related declines in endothelium dependent vasodilation in healthy men. *circulation.* 2000;102:1351-1357.

33. Wessel TR, Arant CB, Olson MB, Johnson BD, Reis SE, Sharaf BL, Shaw LJ, Handberg E, Sopko G, Kelsey SF, Pepine CJ, Merz NB. Relationship of physical fitness vs body mass index with coronary artery disease and cardiovascular events in women.[see comment]. *JAMA.* 2004;292(10):1179-1187.

34. Adams V, Linke A, Krankel N, Erbs S, Gielen S, Mobius-Winker S, Gummert JF, Mohr FW, Schuler G, Hambrecht R. Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation.* 2005;111(5):555-562.

35. Ji LL. Antioxidants and Oxidative Stress in Exercise. *Proc Soc Exp Biol Med.* December 1, 1999 1999;222(3):283-292.

36. Hardin DS, Azzarelli B, Edwards J, Wigglesworth J, Maianu L, Brechtel G, Johnson A, Baron A, Garvey T. Mechanisms of enhanced insulin sensitivity in endurance trained athletes: effects of blood flow and differential expression on GLUT-4 in skeletal muscle. *J Clin Endocrinol Metab.* 1995;80:2437-3446.

37. Vincent HK, Morgan JW, Vincent KR. Obesity exacerbates oxidative stress levels after acute exercise. *Med Sci Sports Exerc.* 2004;36(5):772-779.

38. Matsuoka H. Endothelial dysfunction associated with oxidative stress in human. *Diab. Res. Clin. Pract.* 2001;54(Suppl 2):S65-S72.

39. Goodpaster BH, Katsiaras A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes.* 2003;52(9):2191-2197.

FIGURE LEGEND

Figure 1. The effect of exercise intensity on the FMD response 1hr following the cessation of exercise between groups. Note: A significant group x time interaction exists independent of exercise intensity.

Figure 2. Illustration of the group x time interaction, collapsing for exercise intensity, of FMD normalized to pre-exercise values.

*Significant from pre-exercise values.

†Significantly different from the inactive group.

Figure 3. The effect of acute exercise intensity on plasma concentrations of IL-6 between groups. *Significant from pre-exercise values.

Figure 4. The effect of acute exercise intensity on plasma concentrations of TNF-α between groups.

Table 1. Subject Characteristics

Variables	**Inactive (n=8)**	**Active (n=8)**	***P***
Age, y	56.9±2.6	59.9±2.8	ns
BMI, kg/m^2	29.3±1.0	27.4±0.7	ns
Systolic blood pressure, mm Hg	118.1±2.8	115±4.7	ns
Diastolic blood pressure, mm Hg	79.4±2.7	73.3±2.3	ns
Physical Activity, d/week	0.7±0.3	4.4±0.5	*P*<.001
VO_2peak, ml/kg/min	30.9±1.2	34.2±1.7	ns
Total cholesterol, mg/dL	181.9±12.2	205.1±15.1	ns
HDL cholesterol, mg/dL	48.0±5.5	54.3±5.3	ns
LDL cholesterol, mg/dL	100.6±9.4	129.1±13.0	ns
Triglycerides, mg/dL	166.8±27.0	108.4±15.8	ns
Fasting glucose, mg/dL	95.0±1.6	92.4±3.2	ns
C-reactive protein, mg/L	4.0±2.2	1.5±0.3	ns

Values are mean ± SEM.

Table 2. Physiological variables in response to acute exercise

	Low (25% VO_2peak)		Moderate (50% VO_2peak)		High (75%VO_2peak)	
Variables	**Inactive**	**Active**	**Inactive**	**Active**	**Inactive**	**Active**
Heart rate, bpm	87.8±3.6	75.5±2.9	113.9±5.3	105.4±3.3	143.1±4.3	139.4±4.1
Systolic blood pressure, mm Hg	122.8±4.7	126±6.3	142.4±5.8	132.1±5.6	164.6±6.7	157.6±88.2
Diastolic blood pressure, mm Hg	78.6±4.1	74.1±3.8	74.5±3.3	70.5±2.0	75.1±4.0	69.9±3.0
Rating of perceived exertion	8.0±0.4	7.7±0.2	11.3±0.6	11.4±0.6	14.2±0.5	13.7±0.4
VO_2, ml/kg/min	8.0±0.2	8.7±0.4	15.6±0.7	17.3±0.8	23.3±1.1	25.8±1.3
VO_2, %	26.1±0.5	25.6±0.6	50.4±0.5	50.5±0.4	75.2±1.0	75.4±0.6

Values are mean ± SEM.

Table 3. Pre- and post- exercise artery characteristics by group

Inactive Group (n=8)	Low (25% VO_2peak)		Moderate (50% VO_2peak)		High (75%VO_2peak)	
Variables	**Pre-exercise**	**1 Hr Post**	**Pre-exercise**	**1 Hr Post**	**Pre-exercise**	**1 Hr Post**
Baseline diameters, mm	4.21±0.12	4.15±0.18*	4.08±0.16	4.12±0.18	4.04±0.15	4.30±0.19*
Baseline velocity, cm/s	21.0±3.4	16.5±3.2*	20.5±2.4	18.5±2.4*	22.2±2.6	21.5±3.0*
Peak hyperemic velocity, cm/s	107.0±3.8	99.1±8.0	96.2±8.1	104.4±5.6	98.9±4.0	103.2±5.4
Hyperemic local shear stress, AU	7.2±0.4	6.8±0.7	6.7±0.7	7.3±0.7	6.9±0.4	6.8±0.4

Active Group (n=8)	Low (25% VO_2peak)		Moderate (50% VO_2peak)		High (75% VO_2peak)	
Variables	**Pre-exercise**	**1 Hr Post**	**Pre-exercise**	**1 Hr Post**	**Pre-exercise**	**1 Hr Post**
Baseline diameters, mm	4.16±0.16	4.00±0.14*	4.04±0.15	4.04±0.13	4.02±0.15	4.09±0.17*
Baseline velocity, cm/s	18.5±2.2	14.2±2.1*	18.5±3.6	13.4±1.2*	15.1±1.9	13.6±1.1*
Peak hyperemic velocity, cm/s	83.7±7.7	77.2±6.3*	89.3±7.9	82.5±9.0*	99.0±8.0	78.5±6.5*
Hyperemic local shear stress, AU	5.7±0.6	5.5±0.5	6.3±0.7	5.7±0.6	7.0±0.6	5.5±0.6

* Significant from pre-exercise. Values are mean ± SEM.

Figure 1.

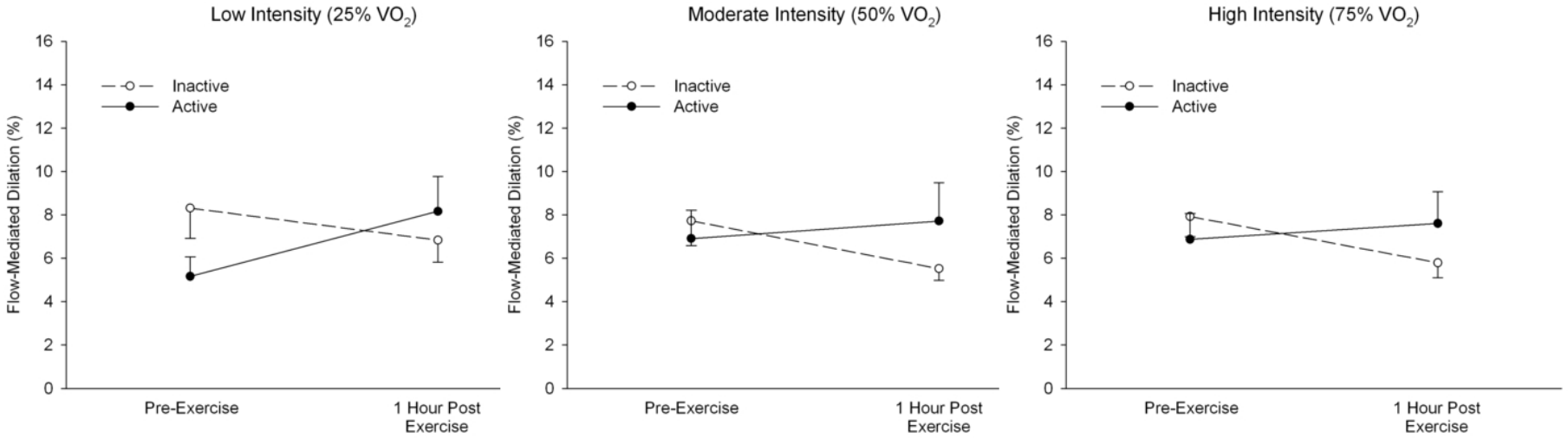

Low Intensity (25% VO2)
Moderate Intensity (50% VO2)
High Intensity (75% VO2)
Flow-Mediated Dilation (%)
16
14
12
10
8
6
4
2
0
Inactive
Active
Pre-Exercise
1 Hour Post Exercise

Figure 2.

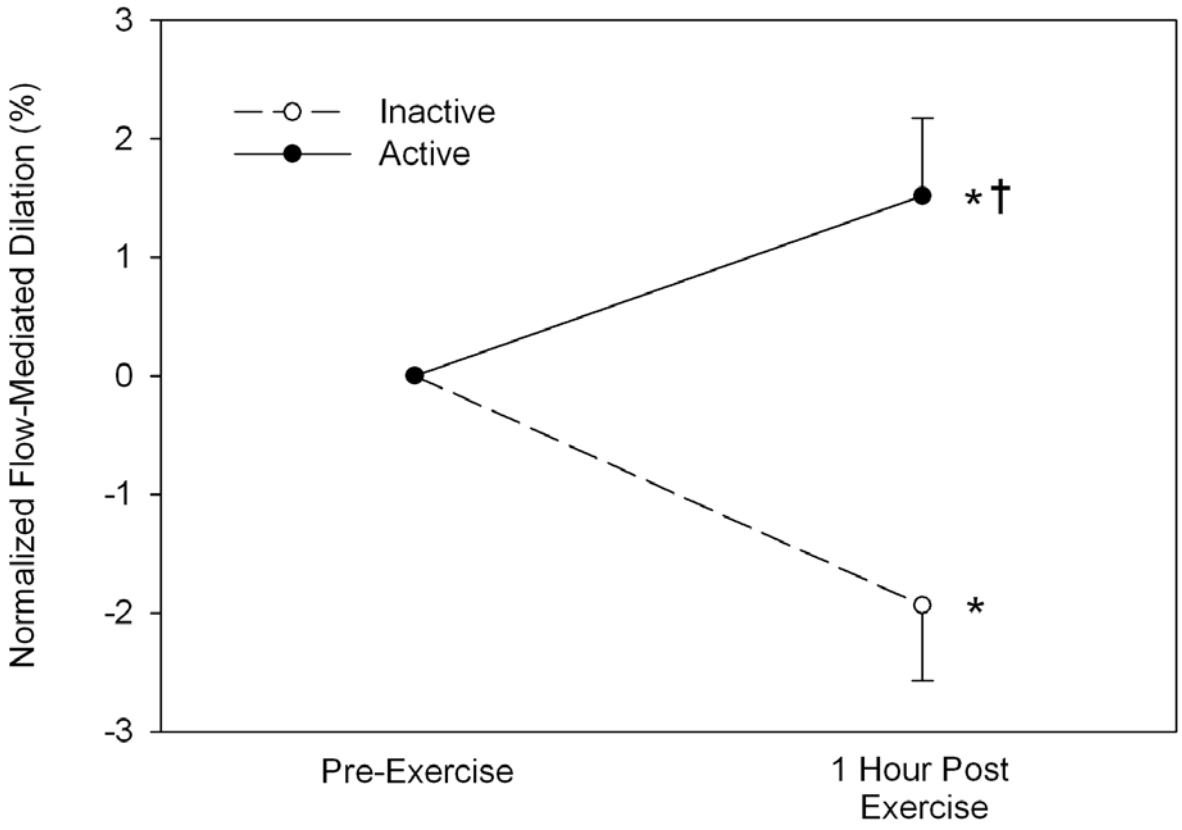

Normalized Flow-Mediated Dilation (%)
3
2
1
0
-1
-2
-3
Inactive
Active
*†
*
Pre-Exercise
1 Hour Post Exercise

Figure 3.

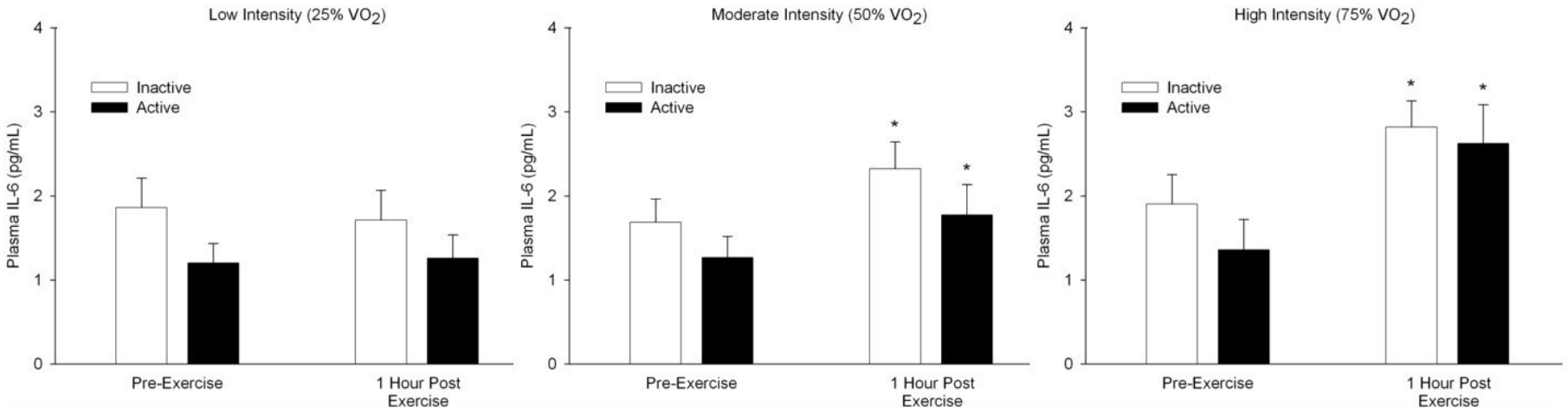
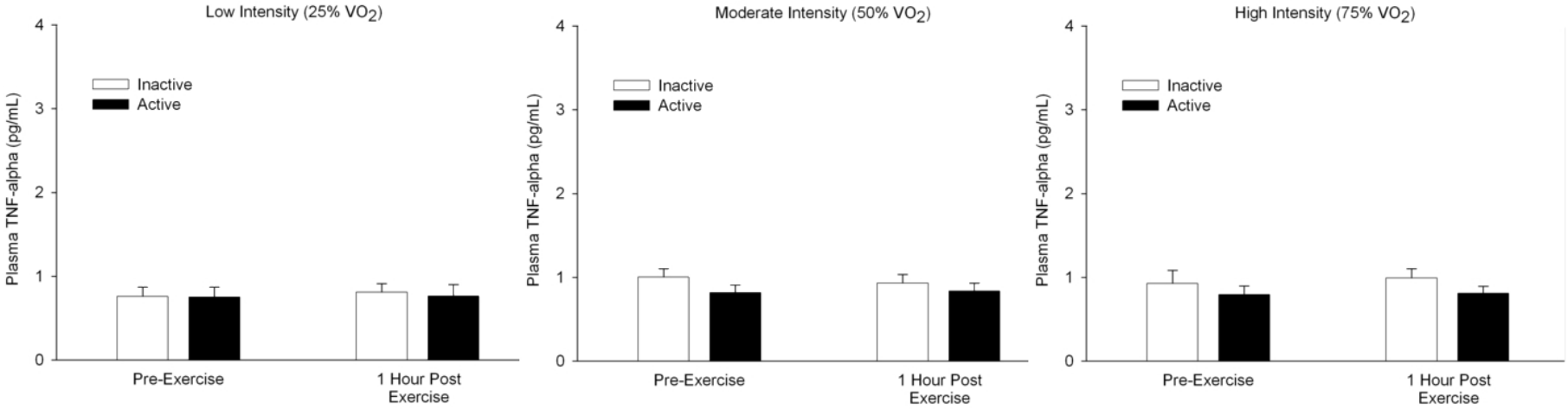

Low Intensity (25% VO2)
Moderate Intensity (50% VO2)
High Intensity (75% VO2)
Inactive
Active
Plasma IL-6 (pg/mL)
0
1
2
3
4
*
Pre-Exercise
1 Hour Post Exercise

Figure 4.


Low Intensity (25% VO2)
Moderate Intensity (50% VO2)
High Intensity (75% VO2)
Plasma TNF-alpha (pg/mL)
Inactive
Active
0
1
2
3
4
Pre-Exercise
1 Hour Post Exercise

APPENDIX A – REVIEW OF LITERATURE

INTRODUCTION TO ENDOTHELIAL FUNCTION

The endothelium is a thin monolayer of cells that lines the walls of all the vessels in the body (figure 1). Until recently, the endothelium was thought to serve as an inert barrier. Instead, the endothelium is suggested to be the largest organ in the body that plays multiple essential homeostatic functions.

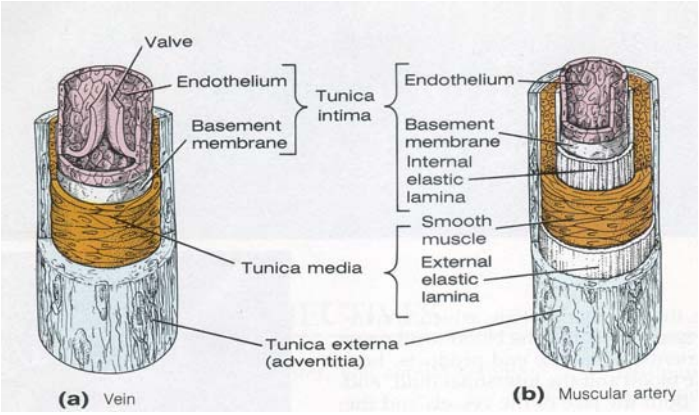


Figure 1. Anatomy of veins and arteries (Spence 1990)

FUNCTIONS OF THE ENDOTHELIUM

Endothelial cells control thrombosis, inhibit leukocyte and platelet adhesion, promote intra-arterial permeability, and regulate vascular smooth muscle tone (17, 94, 119). In addition, there are many substances released from the endothelium that aid in the function of the endothelium which include but are not limited to prostacyclin, nitric oxide (NO), endothelins, endothelial cell growth factors, interleukins, and plasminogen inhibitors. These substances either individually or in conjunction with one another provide different functions illustrated in figure 2, and described below.

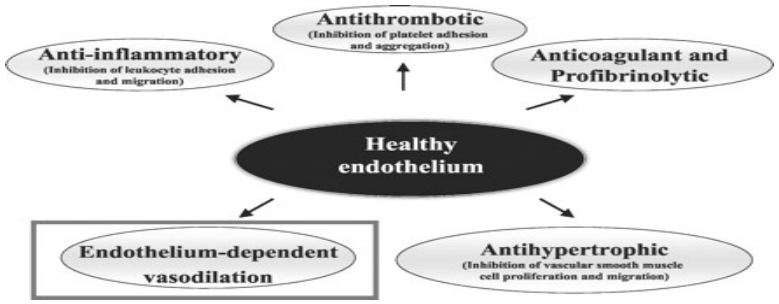


Figure 2. Functions of a healthy endothelium (Landmesser et al. 2004).

Control of Thrombosis and Platelet Adhesion

Endothelial cells are the major site for anticoagulant reactions involving thrombin (28). Prostacyclin is the major endothelial derived anticoagulant that is released from

thrombin, which is the same stimuli that activates platelets, ADP, and ATP (76). Aggregating platelets secrete serotonin and Thromboxane A_2, which cause endothelium independent contraction (95). A healthy endothelium maintains production of prostacyclin which limits the extent of platelet plug formation (53). Furthermore, tissue type plasminogin activator (t-PA) is also an endothelial derived endogenous anticoagulant that is stimulated by norepinephrine, vasopressin, and thrombin to help protect against platelet formation and thrombosis (16).

Leukocyte Adhesion

Activated polymorphonuclear leukocytes (PMN's) contribute to the production of free radical formation and contribute to the pathology of endothelial dysfunction. Nitric oxide released from a healthy endothelium has been postulated to provide a protective chemical barrier against free radicals (95). Prostacyclin has also been shown to interact synergistically with NO to prevent tissue damage by activated PMN's (95). In addition, cell surface adhesion molecules (endothelial leukocyte adhesion molecule-1 (ELAM-1), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)) help regulate leukocyte and platelet adhesion. ELAM-1 is produced in response to an immune injury stimulus to provide a site for neutrophil attachment. Subsequently, VCAM-1 and ICAM-1 are expressed to promote lymphocyte attachment and initiate the local immune facilitation (95).

Permeability

The endothelium serves as the barrier, providing permeability access between the lumen and the intracellular structure of the blood vessel. Low density Lipoprotein (LDL) is constantly migrating back and forth from the blood to the smooth muscle to provide

structural aid in maintaining vascular tone. The release of NO from a normal functioning endothelium promotes inhibition of superoxide anion radicals to oxidize/modify the passing LDL (50). In contrast, a dysfunctional endothelium would produce inadequate concentrations of NO to provide the LDL with protection against oxidization and the permeability of the modified LDL would be restricted.

Regulation of Vascular Smooth Muscle Tone

The regulation of vascular smooth muscle tone assumes a balance between vasoconstriction and vasodilation. Endothelin (ET) is the major class of vasoconstricors which are composed of three different isoforms; however, only ET-1 has been shown to be released from endothelial cells (70). Endothelin-1 is present in healthy populations in low concentrations, has a short half life, and is suggested to play the role of a local vasoconstrictor. In contrast to the limited endothelial derived vasoconstrictors, there are many smooth muscle relaxors. Endothelial derived vasodilators include endothelium-derived hyperpolarizing factor (EDRH), prostacyclin, and NO.

Endothelium-derived hyperpolarizing factor works independent of NO and plays an important role by increasing the K^+ conductance and promoting smooth muscle relaxation (16). Prostacyclin, the first discovered endothelial derived vasoactive substance, is produced via the cyclooxygenase enzyme and also promotes smooth muscle cell relaxation. Just like NO, prostacyclin is chemically unstable and has a limited half-life; however, unlike NO it is produced via stimulation of adenylate cyclase and an increase in intracellular concentrations of cAMP (16). Nitric oxide, a potent vasodilator is the primary molecule responsible for promoting endothelial function is described in detail below.

In 1980, Furchgott and Zawadki (33) discovered one of the most important vasodilators released from the endothelium and named it EDRF. They investigated endothelial function by constricting vessels with norepinephrine and infusing acetylcholine (Ach), which they proposed would induce vasodilation. They were intrigued to discover that the Ach induced vasodilatation was inhibited if the endothelium was damaged. In 1987 Palmer and colleagues (85) took Furchgott and Zawadki's investigation one step further and identified the EDRF as Nitric Oxide (NO).

Nitric Oxide is a very soluble, highly reactive gas that can be produced by certain animal and plant cells from the amino acid L-arginine. The formation of NO through the breakdown of L-arginine produces L-citruline. L-citruline can then be recycled back into L-arginine, which can then produce more NO. Nitric Oxide is a perfect messenger because it is fast, easily passes through cells and membranes and is produced in abundance. Nitric Oxides primary role as an endogenous vasodilator has an intravascular half-life of ~2 milliseconds and extravascular half-life of ~2 seconds (54, 71). Nitric oxide plays an important role in controlling the cardiovascular system by inhibiting platelet-endothelial wall interaction and proliferation of vascular smooth muscle cells (51, 54). The amount of nitric oxide available to provide protective vascular effects is termed NO-bioavailability. An increase in NO-bioavailability decreases the rate of endothelial dysfunction. Endothelial dysfunction has been proposed as the earliest identifiable event in the process of atherosclerosis (120).

PATHOPHYSIOLOGY OF ENDOTHELIAL DYSFUNCTION

There are many mechanical and chemical factors that come in contact with the monolayer of cell lining the vessel wall that may induce damage to the endothelium and

increase the rate of cardiovascular disease. Dysfunction of the endothelium can be defined as an imbalance between relaxing and contracting factors (95) that were discussed in the previous section. Possible contributors of endothelial dysfunction leading to atherosclerosis include, but are not limited to elevated and modified LDL, smoking, free radicals production, hypertension, diabetes mellitus, genetics, elevated plasma concentrations of homocysteine, and chronic infections (92). In addition, NADPH oxidase expression has been linked to vascular reactive oxygen species (ROS) production, in particular superoxide (O_2^-) (63). Reactive oxygen species induce programmed cell death, induce or suppress the expression of many genes, and activate cell signaling cascades (45). Further, ROS contributes to endothelial dysfunction and atherosclerosis in sedentary populations (63).

Atherosclerosis is a progressive inflammatory disease characterized by the accumulation of lipids and fibrous elements in medium to large arteries (65) and has been suggested to be the result of endothelial dysfunction (79, 93). A dysfunctional endothelium allows for oxidized LDL to infiltrate in the vessel wall and initiate the inflammatory response. The immune defense is initiated and macrophages are recruited to engulf the oxidized LDL and facilitate the formation of foam cells. Foam cells are too big to depart the inner vessel space and eventually begin to disrupt the vessel lumen. Once the endothelium becomes dysfunctional, a viscous cycle of atherosclerotic plaque development causes a further increase in endothelial cell dysfunction, which limits the functional properties of the endothelium described above. Unsurprisingly, endothelial dysfunction has been associated with an increase risk for cardiovascular disease in many

different clinical populations (27, 41, 68). The functions of the endothelial cells are tested under many different methodological environments described below.

MEASURES OF ENDOTHELIAL DYSFUNCTION

Arterial health can be measured several different ways either invasively or non invasively. The ultimate invasive procedure involves direct measures of endothelial function within the coronary arteries via femoral artery catheterization; however, this technique is very risky and costly. Another semi invasive procedure utilizes an infusion of Acetylcholine (Ach) into the artery as a vasodilatory agent. The dose dependent infusion of Ach influences vascular tone and the amount of resistance vessel blood flow stimulating the endothelial vasodilatory response. Blood flow, a function of arterial diameter, blood viscosity and velocity, is measured either by ultrasound or strain gauge plethysmography in response to arterial Ach infusion. The increase in diameter in response to the Ach induced increase in flow compared to the baseline diameter is postulated to be an endothelial dependent response, a measure of endothelial function; however there may be reason to think otherwise. As discussed further in this section, the difference in stimulus profiles and its affect on shear stress may influence whether the response will be nitric oxide (NO) - dependent or independent (91) .

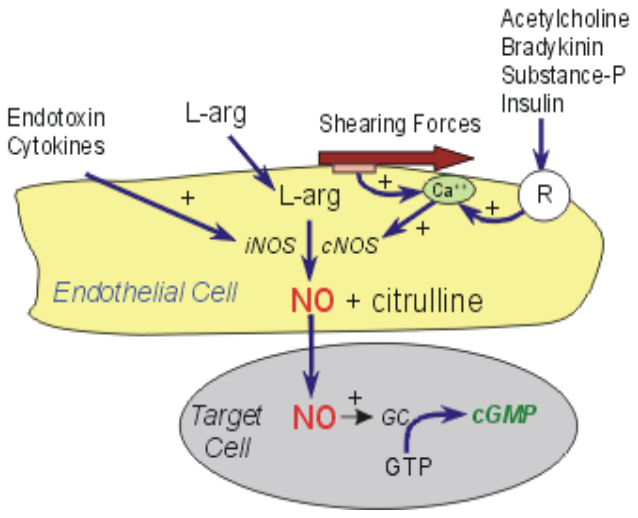


Figure 3. The nitric oxide cyclic GMP pathway.

A rather simple non invasive measure of endothelial function

is obtained by the Pulse Trace® Pulse Contour Analysis (PCA). The PCA tests for an indirect measurement of endothelial function by detecting indexes of arterial stiffness. A compliant artery is suggested to have an enhanced endothelial function, whereas a stiffer artery is suggested to represent a dysfunctional endothelium. The PCA uses a photo-plethysmography transducer with signal conditioning circuitry to obtain an extremely accurate and noise free signal of the Digital Volume Pulse waveform (90). Once again, these pulse waves are utilized as an indirect non invasive measure of the endothelium. The non-invasive methodology most often used and accepted in the literature is the technique of brachial artery flow-mediated dilation (FMD).

The non invasive measurement of brachial artery FMD was first described by Celermajer in 1992 (18). If a variety of conditions are controlled for (23, 91), FMD has been proposed as a non-invasive measurement of NO-dependent endothelial response. To ensure accurate NO dependency, specific conditions to control the shear stress stimulus include but are not limited to the room temperature, diet (fasting or not), previous physical activity, vitamin supplementation, pharmacological interventions (vasoactive medications), the placement of the cuff on the arm (proximal or distal), the duration of occlusion (5 min or longer), smoking, awaking time, and the elimination of actively induced increases in blood flow (23, 91). The brachial artery flow-mediated dilation procedure utilizes ultrasound images of the brachial artery to assess the change in brachial artery diameter in response to occlusion induced reactive hyperemia. Hyperemic flow produces a mechanical force that is exerted parallel (shear) to the long axis of the vessel (82). The increase in shear triggers the G-protein expression of phosphokinase A (mechanisms unknown), signaling an increase of endothelial nitric oxide synthase

(eNOS) expression. L-arginine is upregulated in conjunction with eNOS to produce NO, a potent vasodilator (99). NO diffuses into the tunica media where it upregulates cyclic guanasine triphosphate (cGTP). Guanalyl cyclase converts cGTP to guanasine monophosphate (cGMP) to induce a direct relaxation of the smooth muscle and vasodilation (Figure 3). It is important to note that NO also acts directly to produce smooth muscle relaxation as well. The percent increase in the post reactive hyperemia diameter compared to the baseline diameter is expressed as FMD (%). The measurement of flow-mediated dilation (FMD) via ultrasound has been established as a reliable non-invasive measurement of endothelial function (115) and has been shown to correlate with invasive testing of coronary artery endothelial function (2, 109).

BENEFITS OF EXERCISE ON ENDOTHELIAL FUNCTION

Exercise is indicated in the treatment and prevention of modern disease and may translate to the prevention and/or reversal of endothelial dysfunction. It has long been known that there are protective cardiovascular benefits associated with exercise. Regular physical activity has many benefits including a reduced risk of cardiovascular and all-cause mortality (11). The benefits of exercise have been associated with conditions such as myocardial infarction (122), depression (69), cancer (126), arthritis (52), fibromyalgia (81), osteoporosis (102), and has been perceived as homeopathic therapy for many other diseases and conditions; however, the exact mechanisms of the exercise induced benefits are not fully understood. Long before the advanced measurement of arterial health, the benefits of exercise were investigated on traditional risk factors. Physical training decreases body weight (5), increases insulin sensitivity (46), lowers blood pressure (11), serum triglycerides, and increases serum HDL cholesterol (14). Interestingly, there is

evidence to suggest that exercise still provides physiological benefits even in the presence of well established cardiovascular disease predictors such as smoking and elevated systolic blood pressure and cholesterol (29). In addition, there is evidence to suggest that exercise training does not alter plasma lipids, blood pressure, blood glucose, waist to hip ratio, or body mass index in Type II diabetics and healthy controls, despite significant improvements in both FMD and Ach responses (39). Most importantly, exercise plays a multifaceted beneficial role in the improvement of endothelial function as well as the number one killer in the United States, cardiovascular disease and risk discussed below.

Recent studies suggest the mechanism associated with exercise induced improvements of cardiovascular risk is though improvements in vascular tone (43, 110). Exercise training has been shown to increase the transport of L-arginine (87) and augment blood flow and shear stress, which in turn increases NO production and decreases NO inactivation leading to an increase in NO bioavailability (49), all of which upregulates eNOS activity (124). The increase in L-arginine and eNOS together produce the formation of NO as depicted in Figure 2, which may contribute to the increase in endothelial function.

Regular aerobic endurance exercise decreases the age associated increase in endothelin-1 in women, which may have beneficial effects on the cardiovascular system for prevention of hypertension and atherosclerosis (67). In addition, moderate aerobic training (six, 30-minute sessions per week for 8-weeks) in sedentary subjects resulted in an altered autonomic regulation of HR toward vagal dominance (114), thus reducing the cardiovascular work of the heart.

Exercise also improves adaptations in the muscle itself. Endurance exercise has been associated with an increase in muscle density, mitochondrial enzymes, and glycolytic proteins which allow for an increase in oxidative metabolism and an improvement in maximal oxygen consumption and muscle lactate clearance(13); however, endurance exercise also accelerates the production of free radical formation (24). Furthermore, exercise training reduces vascular expression of NAD(P)H oxidase which results in decreased local ROS generation (1). In addition, exercise has been shown to up regulate antioxidants and decreases indexes of oxidative stress in porcine endothelium (96) as well as in human subjects (32).

ANTIOXIDANTS AND ACUTE EXERCISE

The increase in anti/pro oxidant balance may be adequate to augment endothelial function in response to exercise training in itself; however, there appears to be no change in muscle antioxidant capacity (86), total antioxidant status (121) and capacity (113), or superoxide dismutase (113) following acute exercise which would counteract the increase in OS and explain the augmentation of FMD.

Parise and colleagues (86) investigated the effect of a 12-week progressive resistance exercise training using only one leg on skeletal muscle antioxidant capacity (CuZnSOD; muscle biopsy) in 12 older men (71 ± 7 years of age). Following the 12-week program, the skeletal muscle antioxidant capacity was measured in the non trained leg following an acute bout of the same resistance exercise. Post training, the trained limb demonstrated an increase in antioxidant capacity, whereas no differences in antioxidant capacity were observed following acute exercise in the untrained limb.

Tozzi-Ciancarelli and colleagues (113) investigated total plasma antioxidant capacity (TPAC) and superoxide dismutase (SOD) in 15 healthy sedentary men following an acute bout of strenuous and moderate exercise. There was a decrease in both TPAC and SOD following strenuous acute exercise, whereas no significant changes in TPAC or SOD were observed following moderate acute exercise.

Vincent and colleagues (121) investigated the total antioxidant status (TAS) following either acute resistance (RX) or aerobic (AX) exercise in 28 obese and non-obese subjects. Interestingly, Vincent and colleagues observed an increase and no change in total antioxidant status (TAS) following acute resistance exercise in non-obese and obese subjects, respectively and no change and a decrease in TAS following acute aerobic exercise in non-obese and obese subjects, respectively.

In summary, there is conclusive evidence that supports the increase in antioxidant capacity following chronic exercise; however, there appears to be no change in concentrations of antioxidants measured by TPAC, SOD, or TAS following acute exercise. Further, the similar concentrations of antioxidant status pre and post acute exercise does not provide explanation for the improvement in FMD.

CHRONIC EXERCISE AND ENDOTHELIAL FUNCTION

Within the past decade the effects of exercise on endothelial function has received a vast amount attention. This section will touch upon the dose of chronic exercise evaluating different modes, durations, and intensities. The effect of exercise training on endothelial function has been fully investigated by manipulating mode, frequency and duration of exercise, and using different subject populations.

Kasikcioglu and colleagues (55) report that endothelial dependent vasodilation and reactive hyperemic flow rates are significantly ($p<.05$) higher in endurance athletes than in controls (VO_{2max} = 61.24 $ml \cdot kg^{-1} \cdot min^{-1}$ and 44.49 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively); however, this may not always be the case as discussed later in this section.

In a study utilizing female Yucatan pigs, exercise training influenced both antioxidant and pro-oxidant enzymes to decrease indexes of oxidative stress. Twenty-four 8-12 month old pigs performed 16-19 weeks of treadmill running at 40-50% of maximal oxygen uptake. Antioxidant enzyme SOD activity of the aorta was significantly ($p<.05$) increased, whereas pro-oxidant enzyme NAD(P)H and MDA an index of oxidative stress were reduced ($p<.05$) following exercise training. Endothelial function was not measured directly; however, Rush and colleagues (96) speculate these adaptations may contribute to improved endothelial function. Chronic Exercise training has been associated with reversal of different variations of cardiovascular disease as well as traditional risk factors for cardiovascular disease.

Cardiovascular Disease, Exercise, and Endothelial Function

Endothelial dysfunction occurs early in atherosclerosis in response to cardiovascular risk factors and is primarily the result of reduced nitric oxide bioavailability (123). Patients with cardiovascular disease have demonstrated an attenuated dependent and independent endothelial response compared to controls (2.61±2.91 vs 8.10±7.81% and 17.20±7.93 vs. 23.19 ± 8.89%, respectively) (127). Gokce and colleagues (36) investigated FMD in 40 patients with coronary artery disease (CAD) at baseline and following 10 weeks of supervised cardiac rehabilitation (three 30-minute sessions per week). Exercise was associated with a 29% improvement of functional capacity as well

as a significant increase ($p<.05$) in the posterior tibial artery FMD; however, no change in the brachial artery FMD was observed. In addition, Edwards and colleagues (27) observed 12 weeks of standard cardiac rehabilitation on FMD, oxidative stress, and antioxidant defenses in CAD patients. Exercise training resulted in an improvement of endothelial function via brachial artery FMD (7.9% to 11.1%) and also increased plasma nitrite and nitrate levels, SOD activity and decreased oxidative stress. Coronary endothelial function was assessed using Ach induced vasodilation in 10 patients at baseline and following 4 weeks of supervised exercise. Subjects performed six 10-minute bouts of bicycle ergometer at 80% of HR_{max}. The findings from Hambrecht and colleagues (44) suggest an increase in endothelial dependent vasodilation in both the epicardial coronary vessels as well as the resistance vessels. The improvement in endothelial function via Ach induced dilation was observed by Hambrecht and colleagues (42) in 17 patients with CAD following 10-minutes of rowing and 10 minutes of bicycle ergometer, each three times daily. This improvement in endothelial function was related ($r=.59$) to a shear stress induced phosphorylation of eNOS. Handgrip training, 30-minutes per day, for 8 weeks has also been shown to improve vasodilatory responses to Ach in 12 patients with heart failure (56).

Cardiovascular Risk Factors, Exercise, and Endothelial Function

Traditional cardiovascular risk factors have also been associated with an attenuation of endothelial function (illustrated in Figure 3). Green and colleagues (40) investigated an 8-week circuit training program including resistance

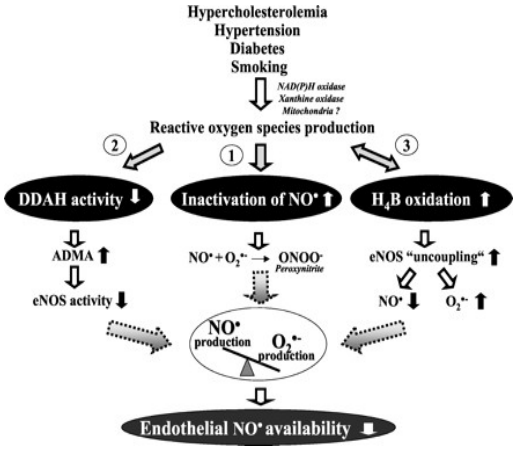


Figure 3. Cardiovascular risk factors mediating endothelial

training, cycle ergometry, and treadmill walking on brachial artery FMD in patients exhibiting cardiovascular risk factors. Findings from this investigation suggest that that short term exercise training improves endothelial dependent nitric oxide-mediated vascular function. In addition, Watts and colleagues (125) investigated the effect of eight weeks of a different circuit training regimen (cycle ergometer at 65-85% HR_{max} and weight training) on the endothelial response via brachial artery FMD in 19 obese (BMI > 34.0 kg/m^2) adolescents. Circuit training not only increased functional capacity, muscular strength, and body composition, but normalized conduit vessel function following training. In contrast to the improvements in EF observed with chronic exercise above, Green and colleagues (39) investigated specific traditional cardiovascular risk factor markers in a diverse clinical population as well as in controls. Eight weeks of circuit training exercise (the same as above) did not significantly ($p>.05$) alter plasma lipids, blood pressure, blood glucose, waist to hip ratio, or BMI; however as described above, exercise did improve brachial artery FMD and Ach induced vasodilatory responses (39). Age is often overlooked as a risk factor for heart disease; although, it has been associated with a decrease in endothelial function (118). The most important age related structural changes may include 1) increased fragmentation and decreased density of elastin in the artery wall, 2) increased collagen content mediated by increased synthesis and decreased turnover, and 3) increased cross-linking of collagen molecules associated with increased advanced glycation end-products (98). In addition, an increase in arterial wall thickness, addition of medial lamellae, and loss of orderly arrangement of elastin in the media may compromise arterial structure and function due to aging (20). These structural changes related to aging (summarized in Table 1) will eventually

increase the risk of clinical consequences. Non structural age related changes such as increased cardiovascular risk factors and decreased NO bioavailability, may also contribute to the reduction in endothelial function; however, there is evidence to suggest possible ways to prevent this age related impairment in previously sedentary middle aged (22-35 yrs) and older (50-76 yrs) apparently healthy men (25).

Table 1. Clinical Consequences of Age Related Structural Changes

↑ Systolic blood pressure
↑ Pulse pressure
↑ Aneurysms and stroke
↑ Endothelial damage
↑ Atherosclerosis
↑ Myocardial Infarction
↑ Coronary artery disease
↑ Peripheral artery disease
↑ Aortic impedance
↑ Left Ventricle wall tension
↑ Peak left ventricle end systolic volume
↑ Arrhythmias
↓ early diastolic filling
↓ Left ventricle systolic reserve
↓ Left ventricle ejection fraction
↓ Arterial baroreflex sensitivity
Adapted from Seals, 2003[96]

Physical activity has been shown to prevent the age related attenuation in NO availability in the elderly (108). Elderly subjects (~65 yrs old) who were regular exercisers displayed a higher acetylcholine induced forearm blood flow than age matched subjects who where sedentary. In a cross-sectional study comparing elderly athletic subjects (exercise at least 45 minutes, 3+ times per week) to age matched sedentary controls, FMD was significantly ($p<.05$) greater in the elderly athletic subjects than their sedentary counterparts (97). Decreases in endothelial function, independent of sex, has been observed using the indirect measurement of endothelial function, PWV. Further, in a crossectional investigation, 150 healthy sedentary men (~25 – 75 years old) were split into age associated quartiles (young, middle age, and old) and carotid artery compliance was measured. Elderly subjects exhibited a 40-50% decrease in compliance compared to young subjects. Similar decreases (~38%) in *aortic* PWV were seen in 50 women ages 26-96 years old (118). The mechanisms associated with the age related impairment in arterial compliance has been attributed to structural and non structural properties of the

arterial wall (98). In addition, higher physical conditioning status, indexed by VO_{2max}, was associated with reduced arterial stiffness measured by aortic PWV (118).

Interestingly, similar results were found in apparently healthy young men of average fitness (19). Brachial artery flow-mediated dilation was performed at baseline and following 10 weeks of aerobic and anaerobic exercise in 25 healthy male military subjects (aged 17-24 years old). Findings from Clarkson and colleagues (19) support an improvement in brachial artery FMD following 10 weeks of 'general' exercise training in young healthy men (19). In addition, O'Sullivan (84) investigated the effect of a 5 week moderate aerobic cycle exercise program in young healthy adult males and report an enhanced endothelium-dependent dilator function measure by FMD. Similar to the results found in young men, Moe and colleagues (74) found that the young women (23.7 ± 2.1 yrs old) also have well preserved endothelial function measured by brachial artery FMD. Interestingly, age matched female endurance trained athletes (VO_{2max} = 60.6± 4.5 ml/kg/min) did not display an improved dilating capacity any further than that of the non-trained females (VO_{2max} = 40.5 ± 5.6 ml/kg/min) (74).

In summary, there is conclusive evidence that physical activity/exercise plays a significant role in regressing or eliminating cardiovascular risk factors; although, there is one investigation that does not support this statement. Exercise seems to improve endothelial dysfunction in populations that have an attenuated endothelial function at baseline. In addition, physical activity and exercise appears to preserve endothelial function in young healthy adults as well as elderly subjects.

ACUTE EXERCISE AND ENDOTHELIAL FUNCTION

A great deal of attention has focused on how and why exercise improves vascular health; however, there is limited research investigating how acute exercise improves endothelial function which is summarized in Table 2. The importance of a single bout of exercise is indisputable. There is strong evidence that an acute bout of exercise can reduce triglycerides, increase high density lipoprotein cholesterol, reduce blood pressure, and improve insulin sensitivity and glucose homeostasis (112). Furthermore, there is evidence to suggest that acute exercise does in fact have an effect on endothelial function.

Harvey and colleagues (48) investigated endothelial function via FMD in pre and postmenopausal women using an exercise stimulus of a single bout of treadmill walking at 60% VO_{2max} for 45 minutes. Baseline measurements and post exercise measurements starting 45- minutes after cessation of exercise were taken in both groups. There was only a significant ($p<.05$) increase in FMD following exercise in the postmenopausal women. Harvey and colleagues speculate that the similar post exercise FMD observed in the pre menopausal women was due to a ceiling effect. A non compromised endothelium maintains its maximum NO bioavailability and the exercise induced increase in shear stress production of NO cannot facilitate any more improvement in endothelial function than already observed. These findings suggest that acute treadmill exercise at 60%VO_{2max} improves endothelial function only in postmenopausal women.

In contrast to the beneficial findings of Harvey and colleagues at moderate intensity, maximal acute exercise induces endothelial dysfunction in claudicants (103). Thirty one patients with intermittent claudication were separated into two groups to participate in exercise either until the claudication pain became intolerable (maximal exercise) or until

the onset of claudication pain (sub-maximal). Flow-mediated dilation was measured at baseline and immediately following cessation of treadmill exercise at a constant speed and increasing the slope 3% every three minutes. A decrease in FMD was only seen following acute symptom limited maximal exercise in claudicants. Seven days following the initial visit, the same protocol as day 1 was initiated with the addition of either a placebo or IV administration of Vitamin C (50mg/min for 20min). Endothelial function following maximal exercise was similar to baseline values with the addition of Vitamin C supplementation. Silvestro and colleagues (103) identified the beneficial role of antioxidant therapy on possible mechanisms associated with the attenuation of FMD following maximal exercise. Vitamin C inhibits the rate of LDL oxidation and upregulates superoxide (O_2^-) scavengering, thus increasing the activation of NO directly. These findings suggest that Vitamin C supplementation prevents acute endothelial dysfunction induced by maximal exercise in patients with intermittent claudication.

The concentrations of urinary nitric oxide and byproducts of NO has been acknowledged and recognized by many (68, 77, 123) as an indirect output measure of endothelial function. Bode-Boger and colleagues (12) investigated the effect of urinary nitrate and cGMP at rest, during, and following acute exercise in 16 men. Sub-maximal exercise was performed on a cycle ergometer for 30 minutes at 60% of their individual maximal work capacity. Urinary nitrate were significantly elevated during the acute exercise and decreased rapidly back to baseline after completion of the exercise bout. Findings from this investigation may support the beneficial role of a single bout of exercise improving ‘endothelial function’ reflected by the increase in nitrate and cGMP;

however, no direct measurement of endothelial function was measured nor was there discussion of the ceiling effect introduced above.

A similar study conducted by Camsari and colleagues (15) investigated the role of Endothelin-1 (ET-1); a potent vasoconstrictor and NO in 25 patients with slow coronary flow (SCF) and 20 control subjects. Plasma samples were collected at baseline and immediately following a standard maximal graduated treadmill exercise test. Endothelin-1 was significantly elevated in the SCF patients and significantly reduced in the control subjects when compared to baseline values. Nitric oxide was significantly elevated in both groups with no difference between the two. The findings of this study suggest that the pathophysiology of SCF patients play a key role in modulating the vasodilatory response following a maximal exercise test and this pathological condition may influence more direct measures of endothelial function.

Kingwell and colleagues (60) investigated arterial compliance following one bout of moderate-intensity cycling in 12 sedentary men. Subjects performed 30-minutes of cycling exercise at 65% of maximal oxygen consumption. Whole body arterial compliance (WBAC) via PWV, aortic flow, and carotid pressure was measured at baseline, 30 minutes and 60 minutes following cessation of exercise. Following a single bout of cycling exercise, WBAC was significantly ($p<.05$) increased immediately (30-minutes) following exercise and returned to baseline values within one hour of exercise cessation. Findings from this study indicate both central and leg arterial compliances independent of changes in mean arterial pressure. Kingwell and colleagues (60) speculate the mechanism related to vasodilation of both the large proximal vessels and the muscle beds for central and peripheral effects, respectively.

Benjamin and colleagues (6) used the Framingham offspring data to compared the FMD of 2142 subjects following a 6-minute walk test (Bruce protocol stages I and II). Endothelial function testing following the 6-minute walk test was improved compared to baseline values. Benjamin and colleagues (6) attribute this improvement in FMD to an NO-dependent increase in forearm blood flow during leg exercises (38).

Investigating the effect of prior exercise on postprandial metabolism and vascular function in obese men, Gill and colleagues (35) performed endothelial function via Ach induced dilation at baseline and ~16-18 hours following 90-minutes of treadmill walking at 50% of VO_{2max}. The results of the postprandial metabolism will not be discussed; however, vascular function was improved ~16-18 hours following a single session of moderate exercise. The specific finding of this investigation is that a 90-minute bout of treadmill walking at moderate intensity improved small vessel vasodilator response in a group of middle aged men.

Gaenzer and colleagues (34) investigated femoral and brachial artery dilation in response to 40-minutes of cycle ergometer exercise in 'healthy' smoking and nonsmoking men. Interestingly, exercise induced hyperemic bloods flow were similar between the two groups. The only diameter change that occurred during exercise was observed in the femoral artery in both groups; however, of the two groups the smokers displayed a diminished percent vasodilation in comparison to the nonsmokers (4.8±1.6% vs. 9.2±1.9%, respectively). Gaenzer and colleagues (34) utilized a non traditional method of FMD, and suggest that the diminished vasodilatory response observed in the smokers was attributable to the endothelial dysfunction induced by smoking.

In summary, there is limited evidence providing the increase in endothelial function following and acute bout of exercise. There is also limited research utilizing the non-invasive methodology of FMD when utilizing the acute paradigm. None of the studies presented in this section offer consistent methodology when measuring endothelial function in the response to acute exercise. It is highly interpretable (based on limited research) that acute exercise improves endothelial function whether measured by byproducts, in the conduit arteries or the resistance arteries. In addition, the doses of exercise used in the presented investigations are different and may play an important role when investigating the effects of acute exercise on endothelial function. There also remains one major unanswered question: There are many reasons why exercise training may improve endothelial function, but why does endothelial function improve after a single bout of exercise? Possible insight to the answer of this question can be found in the following sections.

Table 2. Summary of studies on acute exercise and endothelial function

Author	Subjects	Mode	Intensity (% of VO2max)	Duration (min)	Endothelial Function Method	Findings
Benjamin et al., 2004 (6)	2142 subjects	TM		6-min walk	FMD	↑ FMD imediately following
Bode-Boger et al., 1994 (12)	Healthy Men	Cycle	60	30	Urinary Nitrate and cGMP	↑ in urinary nitrate and cGMP during ↔ in urinary nitrate and cGMP immediately following
Camsari et al., 2003 (15)	SCF Healthy Controls	TM	Maximal Test	Max Test	ET-1 and NO	↑ ET-1 in SCF patients immediately following ↓ ET-1 controls immediately following ↑ NO both groups immediately following
Gaenzer et al., 2001 (34)	Healthy smokers Healthy nonsmokers	Cycle	100-150 watts	40	Exercise induced hyperemia/vasodilation	↑ femoral artery diameter during exercise ↔ brachial artery diameter during exercise
Gill et al., 2004 (35)	Obese Men	TM	50	90	Ach dilation	↑ Ach induced dilation 16-18h following exercise
Harvey et al., 2005 (48)	Pre menopausal W Post menopausal W	TM	60	45	FMD	↑ FMD Post menop. W 1hr following ↔ FMD Pre menop. W 1hr following
Kingwell et al., 1997 (60)	Healthy Men	Cycle	65	30	WBAC	↑ WBAC imediately and returned to baseline within 1hr
Silvestro et al., 2002 (103)	Claudicants	TM	Symptom Limited Maximum	Symptom Limited	FMD	↓ FMD w/o Vit C immediately following ↔ FMD with Vit C immediately following

Subjects- Slow coronary flow (SCF), women (W)
Mode- Treadmill (TM), Cycle ergometer (Cycle)
Endothelial Function- Flow-mediated dilation (FMD), cyclic guanasine monophosphate (cGMP), endothelin-1 (ET-1), acetylcholine (Ach), whole body arterial compliance (WBAC).

DOSE RESPONSE OF EXERCISE ON ENDOTHELIAL FUNCTION

The dose of exercise can be broken up into four different elements; mode, intensity, frequency, and duration. Each element may contribute in different ways in identifying the effect of the dose response relationship of exercise on endothelial function. High intensity training has been associated with greater increases in performance goals where as moderate intensity exercise is often recommended for health related benefits (57, 117). The research investigating the dose response of acute and chronic exercise and its relationship with endothelial function is limited and presented below.

Forjaz and colleagues (31) investigated the role of exercise intensity on post exercise hypotension (PEH) in 23 normotensive subjects. Subjects performed 45-minutes of cycle ergometer exercise at 30%, 50% and 75% of VO_{2peak}. Their findings suggest that PEH is greater and longer following more intense exercise (50% and 75% VO_{2peak}). In addition, Moriguchi and colleagues (78) found an improvement in brachial artery flow mediation following low frequency mild aerobic exercise (60-minutes of 50% VO_{2max}, 2 times per week) for 12 weeks in mild hypertensive subjects.

Bergholm and colleagues (7) investigated endothelial function via Ach induced vasodilation, in nine healthy males at baseline and following three months of marathon training (~70%-80% of VO_{2max}). The intense exposure to repeated bouts of free radical formation impaired endothelial function and deceased circulating antioxidant concentrations in these athletes.

An epidemiological study was conducted by Tanasescu and colleagues (111), looking at a cohort of 44,452 men. Exercise intensities were analyzed separately and related to a reduced risk of coronary heart disease. Further analysis and findings of this study

suggest that *certain* moderate intensity activities/exercise (4-6 METS; walking, running, and weight training) were associated with the greatest relative risk reduction, where as cycling, swimming and racquet sports were not (111). In addition, King and colleagues (59) analyzed C-reactive protein (CRP, and inflammatory biomarker) data from 4072 subjects and found a significant relationship between different types of physical activity and inflammation. Regular jogging and aerobic dancing had a lower likelihood of elevating CRP than that of cycling and swimming; however, exercise intensity and duration were not documented and these differences may be a result of the dose of exercise presented.

In addition, Lee and colleagues (64) investigated the role of moderate intensity exercise relative to the individuals exercise capacity. Subjects (n=7337) reported their actual activities and rated their perceived exertion (RPE) of exercise intensity using the Borg Scale. Findings suggest that RPE was a strong predictor of lower cardiovascular disease. More specifically, there appears to be a dose response relationship, with higher perceived intensities associated with greater decrements in coronary heart disease rates.

Kemi and colleagues (57) investigated the relative effectiveness of 10-weeks of high and moderate exercise intensity on cellular and cardiovascular function in rats. Sprague-Dawley rats preformed treadmill running exercise at either a high (85%-90% VO_{2ma}) or moderate (65%-70% VO_{2max}) intensity for one-hour per day, five days per week. Following 10-weeks of training, carotid artery endothelial function improved similarly with both intensities. Findings from this investigation suggest that cardiovascular adaptations to training are intensity dependent and moderate intensity exercise improved endothelial function to nearly the full effect.

Goto and colleagues (37) investigated the effects of three different intensities of exercise on endothelial function in humans. Twenty six apparently healthy Japanese men were divided into three groups to perform either mild (25% VO_{2max}), moderate (50% VO_{2max}), or high intensity cycle ergometer exercise, for 30-minutes, 5-7 times per week, for 12 weeks. Endothelial function via Ach induced vasodilation was performed at baseline and following exercise training. Endothelial function was significantly augmented in the moderate intensity group; however, no change was observed in the mild or high intensity group. Findings from this investigation suggest that the increase in endothelial dependent dilation following moderate intensity cycling may be due to the increased production of nitric oxide, where as the high intensity exercise increased oxidative stress.

In summary, research supports that most exercise induced benefits appear to occur following moderate intensity activity/exercise. In addition, certain modes of exercise may be more beneficial than others. Most of the literature utilizing the elements of the dose response of exercise involves epidemiological investigations which may be associated with vast amounts of subjectivity-reported error. There is limited data controlling for exercise intensity and its effect on endothelial function. Furthermore, there are no studies to my knowledge that investigate the dose response of acute exercise on endothelial function measured by brachial artery flow mediated dilation.

BIOMARKERS, EXERCISE AND ENDOTHELIAL FUNCTION

There is a strong relationship between inflammation and endothelial dysfunction. There is also a strong relationship between exercise induced inflammation and endothelial function. As depicted in figure 4 below, inflammatory signals activate the

release of IkB (not seen in the diagram) which allows the translocation of nuclear factor kappa beta (NF-kB) to migrate inside the nucleus and bind to the kB sites in the promoter region of the DNA. Subsequently, messenger RNA is expressed promoting the production of inflammatory proteins. This next section will attempt to identify the exercise induced response of two specific cytokines (TNF-alpha and Interleukin-6) and

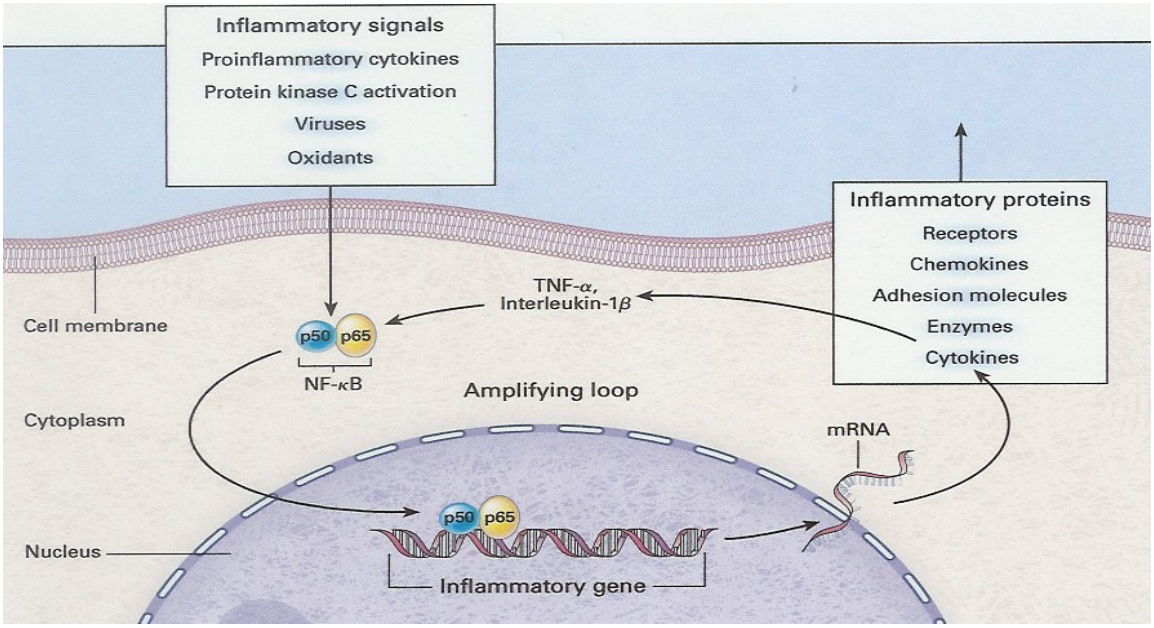


Figure 4. Schematic diagram of inflammatory protein release in response to activation signals (Barnes and Karin 1997).

their relationship to the outcome variable of interest; endothelial function.

Tumor Necrosis Factor- Alpha

Tumor Necrosis Factor-alpha (TNF-α) is a pro inflammatory cytokine produced primarily by macrophages and cytoxic CD8 and CD4 T cells, that also plays a positive feedback role to increase its own production and stimulate the acute phase proteins (systemic inflammatory markers, ie. CRP) and cellular components of the immune system (72). Other functions of TNF-α include; decrease glucose uptake in working muscles (107), recruitment of additional macrophages, direct killing of infected cells,

localized inflammation, and increases in core temperature (66). In addition, TNF-α is a mediator of ROS (10) by stimulating the generation of superoxide ion via activation of NAD(P)H oxidase (58), which may diminish NO bioavailability (47) and decrease endothelial function. Furthermore, TNF-α is postulated to be the first responder (26) of the cytokine family in mediating ROS (10) and may reflect a change associated with exercise induced endothelial function; however, this needs to be investigated further.

Bhagat and Vallance (9) have shown that infusion of TNF-α impairs endothelial function in healthy subjects. In addition, consistent elevations in TNF-α results in loss of its anti- tumor activity producing anti-TNF antibodies which may result in additional cell necrosis (8). There is evidence suggesting patients with attenuated endothelial function have elevated plasma concentrations of TNF-α (21, 62). Studies by Anker and colleagues (3) further support the role of TNF-α induced endothelial dysfunction by contributing to skeletal muscle fatigue/weakness in CHF patients. There is also evidence that suggests combined endurance/resistance exercise training (60-minutes per day, three times per week) decreases plasma TNF-α receptor expression in patients with chronic heart failure (21). In addition, plasma TNF-α was reduced following 12-weeks of cycle ergometer exercise for 30-minutes per day, three days per week in CHF patients (62).

Natelson and colleagues (80) observed a decrease in direct gene expression of TNF-α following a maximal graded exercise stress test; however, there was no change in plasma concentrations of TNF-α. Similar findings of no change in plasma TNF-α were observed following long duration exercise (3 hours of cycling and inclined walking) at 60-65% VO_{2max} (105), 60-minutes of moderate cycle ergometry exercise (~75% of VO_{2max}) (116),

and six hours of endurance running in well trained athletes (26). It is important to note that the dose response of exercise; specifically, intensity and duration, plays a key role in the reduction and elevation of TNF-α. In addition, the population studied may also influence the results of TNF-α following exercise.

Interestingly, in a subsequent analysis of three hours of endurance exercise, Starki and colleagues (105) found that long duration endurance exercise inhibits the endotoxin-induced TNF-α production. They suggest that the exogenous infusion of endotoxin mediated TNF-α production is blunted by an exercise induced mechanism and there appears to be a down regulation of TNF-α production. They further postulate that the exercise mediates an increase in anti-inflammatory activity through an Interleukin-6 mechanism.

Interleukin-6

Interleukin-6 (IL-6) is also a key player of the immune system and is the major cytokine responsible for mediating the release of acute phase proteins from the liver (66); although, it is typically the last inflammatory cytokine to respond (26). Interleukin-6 may be produced by many cells, primarily monocytes, activated lymphocytes, and adipose tissue. Interleukin-6 can also be released from the liver and the gut in non exercise induced stress (30). Interleukin-6 has been classified as both a pro- and anti-inflammatory cytokine; however, the current view accepts the primary role as having anti-inflammatory effects (89). Interleukin-6 acts to stimulate the hypothalamus-pituitary axis in response to inflammation, promotes osteoclastogenesis, and influences intermediary metabolism (73). Elevations in IL-6 concentrations may be due to increases in immune activity (101), unrelated inflammation (22), catecholamine production (105),

and exercise (73). The exercise-induced increase in IL-6 concentrations has been suggested to be a consequence of an immune response do to local damage in the working muscle (83), until recently debated.

Starkie and colleagues (106) have demonstrated that the immune cells are not the source of the increase in plasma IL-6 during exercise. Furthermore, Febbraio and colleagues (30) suggest the production of IL-6 in response to exercise is not an exercise induced immune response, it's a direct release from the muscle contraction itself. They refer to IL-6 as a myokine, a cytokine released from the skeletal muscle itself. The investigations from Pedersen and colleagues (88) support this phenomenon by suggesting that IL-6 is linked more so with metabolism (exercise) than it is with inflammation. Furthermore, the production of IL-6 during exercise is related to the intensity and duration of the exercise (89).

Ullum and colleagues (116) report an increase in IL-6 in response to one-hour of cycle ergometry at 75% of maximal oxygen intake. In addition a 3 hour combination of cycle ergometry and treadmill running at 60-65% VO_{2max} increased IL-6 concentration greater than 18 fold compared to baseline values (75). Increases in IL-6 in response to cycling, treadmill, rowing, and eccentric exercise have all been reported (100). In addition, there appears to be a dose response relationship of amount of plasma IL-6 observed and exercise intensity (40% - 90%) with the greatest increase occurring at a relative intensity of ~75% (100).

There is conclusive evidence that IL-6 is released in response to all types, modes, and durations of exercise; however, the remaining unanswered question of why it is released is debated. During exercise there may be an increase in IL-6 from muscle to increase

lipolysis and fat oxidation, but Pedersen and colleagues (88) report that IL-6 is not the sole mediator of these outcomes. IL-6 has also been shown to impair TNF-a expression in cardiac muscle (107) and skeletal muscle (89); consequently, the augmentation of endothelial function associated with acute and chronic exercise may be associated with the IL-6 mediated TNF-a suppression.

In summary, it appears that TNF-α and IL-6 are tightly linked. TNF-α is released in response to high levels of stress hormones (ie. catecholamines and cortisol), whether it be physical stress or psychosocial stress. Furthermore, chronic exercise has been shown to decrease TNF-α concentrations in clinical populations. There is concrete evidence that there are exercise associated increases in plasma concentrations of IL-6; however, there appears to be no change in plasma TNF-α in response to acute exercise unless the dose consists of high intensity and long duration. IL-6 has been classified as both an anti and pro inflammatory cytokine; however, recent evidence suggests its primary responsibility is an anti-inflammatory myokine. It still remains unclear whether IL-6 acts directly as an anti-inflammatory mediator or whether it acts to stimulate other anti-inflammatory cytokines (ie. IL-1ra and IL-10). In addition, it is unclear if the elevated concentrations of IL-6 in response to exercise directly influence the endothelial response. The increase in concentrations of IL-6 in response to exercise may be a factor in controlling the pro-inflammatory response, thus limiting the TNF-α response, resulting in an improvement of endothelial function. Or is the major production of IL-6 produced in conjunction with depleted muscle glycogen, and does not play a contributing role in the endothelial response at all? Needless to say, further investigations are warranted to identify this phenomenon.

OVERALL CONCLUSION

Over the past 20 years, research has discovered essential physiological functions from a mono layer of cells known as the endothelium. A dysfunctional endothelium has been identified to be the first indicator of several pathological diseases. More and more research is focusing on exercise training as a non-pharmacological agent in providing maintenance or improvements in endothelial function, yet there is limited research utilizing an acute exercise model. There is no doubt that exercise in general improves endothelial function; however, the mechanisms associated with this improvement are not fully understood. There are several biomarkers that need to be investigated to assist in identifying the possible mechanisms associated with the exercise mediated improvements in endothelial function. It may seem intuitive to assess markers of oxidative stress and/or antioxidants to help explain the improvement of FMD following chronic exercise; however, there is conclusive evidence that suggests an increase in oxidative stress along with no change in antioxidant status following acute exercise. This evidence would not support the augmentation of endothelial function. So what are the possible mechanisms associated with the improvement in endothelial function following acute exercise? This review has supported investigating the combination of TNF-alpha and IL-6 together following acute exercise. The relationship of these two biomarkers may provide insight into linking the possible physiological mechanisms associated with the improvement in FMD following a single exercise session.

REFERENCES

1. Adams, V., A. Linke, N. Krankel, S. Erbs, S. Gielen, S. Mobius-Winker, J. F. Gummert, F. W. Mohr, G. Schuler, and R. Hambrecht. Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation*. 111:555-562, 2005.
2. Anderson, T. J., A. Uehata, and M. D. Gerhard. Close relationship of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 26:1235-1241, 1995.
3. Anker, S. D., M. Volterrani, K. R. Egerer, C. V. Felton, W. J. Kox, P. A. Poole-Wilson, and A. J. A. Coats. Tumor necrosis factor alpha as a predictor of impaired peak leg blood flow in patients with chronic heart failure. *QJM*. 91:199-203, 1998.
4. Barnes, P. J. and M. Karin. Nuclear factor-kB- A pivital transcription factor in chronic inflammatory diseases. *N Engl J Med*. 336:1066-1071, 1997.
5. Bassuk, S. S. and J. E. Manson. Epidemiological evidence for the role of physcial activity in reducing risk of type 2 diabetes and cardiovascular disease. *J Appl Physiol*. 99:1193-1204, 2005.
6. Benjamin, E., M. Larson, M. J. Keys, G. F. Mitchell, R. S. Vasan, J. F. Keaney, B. T. Lehman, S. Fan, E. Osypiuk, and J. A. Vita. Clinical correlates and heritability of flow-mediated dilation in the community: The framingham heart study. *Circulation*. 109:613-619, 2004.
7. Bergholm, R., S. Makimattila, M. Valkonen, M. Liu, S. Lahdenpera, M. Taskinen, A. Sovijarvi, P. Malmberg, and H. Yki-Jarvinen. Intense physical training

decreases circulating antioxidants and endothelium-dependent vasodilation in vivo. *Atherosclerosis*. 145:341-349, 1999.

8. Beutler, B., D. Greenwald, J. Hulmes, M. Chang, Y. Pan, J. Mathison, R. Ulevitch, and A. Cerami. Identity of tumor necrosis factor and the macrophage secreted factor cachectin. *Nature*. 316:552-554, 1985.

9. Bhagat, K. and P. Vallance. Inflammatory cytokines impair endothelial-dependent dilation in human veins in vivo. *Circulation*. 96:3042-3047, 1997.

10. Bilsborough, W., G. O'Driscoll, K. Stanton, R. Weerasooriya, L. Dembo, R. Taylor, and D. Green. Effect of lowering tumor necrosis factor-a on vascular function in type II diabetes. *Clin Sci*. 106:163-169, 2002.

11. Blair, S. N., H. W. Kohl, R. S. Paffenbarger, D. G. Clark, and K. H. Cooper. Physical fitness and all cause mortality. *JAMA*. 262:2395-2401, 1989.

12. Bode-Boger, S. M., R. H. Boger, E. P. Schroder, and J. C. Frolich. Exercise increases systemic nitric oxide production in men. *J Cardiovasc Risk*. 1:173-178, 1994.

13. Brooks, G. A., T. D. Fahey, T. P. White, and K. M. Baldwin. *Exercise Physiology*. 3 ed. Mountain View: Mayfield Publishing Company, 2000

14. Brownwell, K. D. and P. S. Bachorik. Changes in plasma lipids and lipoprotein levels in mena nd women after a program of moderate exercise. *circulation*. 65:477-484, 1982.

15. Camsari, A., H. Pekdemir, D. Cicek, G. Polat, M. N. Akkus, O. Doven, G. Cin, T. Katircibasi, and T. Parmaksiz. Endothelin-1 and nitric oxide concentrations and

their response to exercise in patients with slow coronary flow. *circ J*. 678:1022-1028, 2003.

16. Celermajer, D. Endothelial dysfunction: Does it matter? Is it reversible? *J Am Coll Cardiol*. 30:325-333, 1997.

17. Celermajer, D. Endothelial dysfunction: does it matter? is it reversible? *Journal of American College of Cardiology*. 30:325-333, 1997.

18. Celermajer, D. S., K. E. Sorensen, and V. M. Gooch. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 340, 1992.

19. Clarkson, P., H. E. Montgomery, M. J. Mullen, A. E. Donald, A. J. Powe, T. Bull, M. Jubb, M. World, and J. E. Deanfield. Exercise training enhances endothelial function in young men. *J Am Coll Cardiol*. 33:1379-1385, 1999.

20. Cliff, W. *Aging and arterial structure*. New York: Plenum, 1981, 635-665.

21. Conraads, V. M., P. Beckers, J. Bosmans, L. S. DeClerck, W. J. Stevens, C. J. Vrints, and D. L. Brutsaert. Combined endurance/resistance training reduces plasma TNF-alpha receptor levels in patients with chronic heart failure and cornary artery disease. *Eur Heart J*. 23:1854-1860, 2002.

22. Corpeleijn, E., W. H. M. Saris, E. H. J. M. Jansen, P. M. H. J. Roekaerts, E. J. M. Feskens, and E. E. Blaak. Postprandial Interleukin-6 Release from Skeletal Muscle in Men with Impaired Glucose Tolerance Can Be Reduced by Weight Loss. *J Clin Endocrinol Metab*. 90:5819-5824, 2005.

23. Corretti, M. C., T. J. Anderson, E. J. Benjamin, D. Celermajer, F. Charbonneau, M. A. Creager, J. Deanfield, H. Drexler, M. Gehard-Herman, D. Herrington, P.

Vallance, J. Vita, and R. Vogel. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery. *J Am Coll Cardiol*. 39:257-265, 2002.

24. Davies, K. J., A. T. Quintanilha, G. A. Brooks, and L. Packer. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun*. 107:1198-1205, 1982.

25. DeSouza, C. A., L. F. Shapiro, C. M. Clevenger, F. A. Dinenno, K. D. Monahan, H. Tanaka, and D. R. Seals. Regular aerobic exercise prevents and restores age-related declines in endothelium dependent vasodilation in healthy men. *circulation*. 102:1351-1357, 2000.

26. Drenth, J. P., S. H. Van Uum, M. Van Deuren, G. J. Pesman, J. Van der Ven-Jongekrijg, and J. W. Van der Meer. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production. *J Appl Physiol*. 79:1497-1503, 1995.

27. Edwards, D. G., R. S. Schofield, S. L. Lennon, G. L. Pierce, W. W. Nichols, and R. W. Braith. Effect of exercise training on endothelial function in men with coronary artery disease. *Am J Cardiol*. 93:617-620, 2004.

28. Esmon, N. L. Thrombomodulin. *Semin Thromb Hemost*. 13:454-463, 1987.

29. Farrell, S. W., J. B. Kampert, H. W. Kohl, C. E. Barlow, C. A. Macera, R. S. Paffenbarger, L. W. Gibbons, and S. N. Blair. Influences of cardiorespiratory fitness levels and other predictors on cardiovascular disease mortality in men. *Med Sci Sports Exerc*. 30:899-905, 1998.

30. Febbraio, M. A. and B. K. Pedersen. Contraction induced myokine production and release: Is skeletal muscle an endocrine organ. *Exerc Sport Sci Rev*. 33:114-119, 2005.

31. Forjaz, C. L., C. G. Cardoso, C. C. Rezk, D. F. Santaella, and T. Tinucci. Postexercise hypotension and hemodynamics: the role of exercise intensity. *J Sports Med and Phys Fit*. 44:54-62, 2004.

32. Franzoni, F., L. Ghiadoni, F. Galetta, Y. Plantinga, V. Lubrano, Y. Huang, G. Salvetti, F. Regoli, S. Taddei, S. Santoro, and A. Salvetti. Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men. *AJH*. 18:510-516, 2005.

33. Furchgott, R. F. and J. V. Zawadzki. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 288:373-376, 1980.

34. Gaenzer, H., G. Neumayr, P. Marschang, W. Sturm, R. Kirchmair, and J. R. Patsch. Flow mediated vasodilation of the femoral and brachial artery induced by exercise in healthy non smoking and smoking men. *J Am Coll Cardiol*. 38:1313-1319, 2001.

35. Gill, J. M. R., A. Al-Mamari, W. R. Ferrell, S. J. Cleland, C. J. Packard, N. Sattar, J. R. Petrie, and M. J. Caslake. Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol*. 44:2375-2382, 2004.

36. Gokce, N., J. A. Vita, D. S. Bader, D. L. Sherman, L. M. Hunter, M. Holbrook, C. O'Malley, J. F. Keany, and G. J. Balady. Effect of exercise on upper and lower

extremity endothelial function in patients with coronary artery disease. *Am J Cardiol*. 90:124-127, 2002.

37. Goto, C., Y. Higashi, M. Kimura, K. Noma, K. Hara, K. Nakagawa, M. Kawamura, K. Chayama, M. Yoshizumi, and I. Nara. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans. Role of endothelium-dependent nitric oxide and oxidative stress. *Circulation*. 108:530-535, 2003.

38. Green, D., C. Cheetham, L. Mavaddat, K. Watts, M. Best, R. Taylor, and G. O'Driscoll. Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *Am J Physiol Heart Circ Physiol*. 283:H899-907, 2002.

39. Green, D. J., J. H. Walsh, A. Maiorana, M. J. Best, R. R. Taylor, and J. G. O'Driscoll. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *Am J Physiol Heart Circ Physiol*. 285:H2679-H2687, 2003.

40. Green, D. J., J. H. Walsh, A. Maiorana, V. Burke, R. R. Taylor, and J. G. O'Driscoll. Comparison of resistance and conduit vessel nitric oxide-mediated vascular function in vivo: effects of exercise training. *J Appl Physiol*. 97:749-755, 2004.

41. Guerci, B., A. Kearney-Schwartz, P. Bohme, F. Zannad, and P. Drouin. Endothelial dysfunction and type 2 diabetes. *Diabetes Metab*. 27, 2001.

42. Hambrecht, R., V. Adams, S. Erbs, A. Linke, N. Krankel, Y. Shu, Y. Baither, S. Gielen, H. Thiele, J. F. Gummert, F. W. Mohr, and G. Schuler. Regular physical

activity improves endothelial function in patients with coronary artery disease by increasing phyosphorylation of endothelial nitric oxide synthase. *Circulation*. 107:3152-3158, 2003.

43. Hambrecht, R., E. Fiehn, C. Weigl, S. Gielen, C. Hamann, R. Kaiser, J. Yu, V. Adams, J. Niebauer, and G. Schuler. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation*. 98:2709-2715, 1998.

44. Hambrecht, R., A. Wolf, S. Gielen, A. Linke, J. Hofer, S. Erbs, N. Schoene, and G. Schuler. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med*. 342:454-460, 2000.

45. Hancock, J. T., R. Desikan, and S. J. Neill. Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Transac*. 29:345-350, 2001.

46. Hardin, D. S., B. Azzarelli, J. Edwards, J. Wigglesworth, L. Maianu, G. Brechtel, A. Johnson, A. Baron, and T. Garvey. Mechanisms of enhanced insulin sensitivity in endurance trained athletes: effects of blood flow and differential expression on GLUT-4 in skeletal muscle. *J Clin Endocrinol Metab*. 80:2437-3446, 1995.

47. Harrison, D. G. Cellular and molecular mechanism of endothelial cell dysfunction in type II diabetes. *J Clin Invest*. 100:2153-2157, 1997.

48. Harvey, P. J., B. L. Morris, T. Kubo, P. E. Picton, W. S. Su, C. F. Notarius, and J. S. Floras. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *Hypertension*. 23:285-292, 2005.

49. Higashi, Y., T. Oshima, and R. Ozono. Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension*. 30:252-258, 1997.

50. Hogg, N., B. Kalyanaraman, J. Joseph, A. Struck, and S. Parthasarathy. Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. *FEBS Lett.* 334:170-174, 1993.

51. Huang, P. L. Mouse Model of Notric Oxide Synthase Deficiency. *J Am Soc Nephrol*. 11:S120-S123, 2000.

52. Hurley, M. V., H. L. Mitchell, and N. Walsh. In osteoarthritis, the psychosocial benefits of exercise are as important as physiological improvements. *Exerc Sport Sci Rev*. 31:138-143, 2003.

53. Jaffe, E. A. Cell biology of endothelial cells. *Hum Pathol*. 18:234-239, 1987.

54. Kaplan, N. *Kaplans Clinical Hypertension*. Philidelphia: Lippincott Williams and Wilkins, 2002

55. Kasikcioglu, E., H. Oflaz, H. A. Kasikcioglu, A. Kayserilioglu, S. Umman, and M. Meric. Endothelial flow mediated dilation and exercise capacity in highly trained endurance athletes. *Tohoku J of Exp Med.* 205:45-51, 2005.

56. Katz, S. D., J. Yuen, R. Bijou, and T. H. LeJemtel. Training improves endothelium-dependent vasodilation in resistance vessels of patients with heart failure. *J. Appl. Physiol.* 82:1488-1492, 1997.

57. Kemi, O. J., P. M. Haram, J. P. Loennechen, J. Osnes, T. Skomedal, U. Wisloff, and O. Ellingsen. Moderate vs high exercise intensity: differential effects on

aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovasc Res*. 67:161-172, 2005.

58. Keulenaer, G. W. D., R. W. Alexander, M. Ushio-Fukai, N. Ishizaka, and K. K. Griendling. Tumour necrosis factor a activates a p22phox-based NADH oxidase in vascular smooth muscle. *J Bichem*. 329:653-657, 1998.

59. King, D. E., P. Carek, A. G. M. III, and W. Pearson. Inflammatory markers and exercise: differences related to exercise type. *Med Sci Sports Exerc*. 35:575-581, 2003.

60. Kingwell, B. A., K. L. Berry, J. D. Cameron, G. L. Jennings, and A. M. Dart. Arterterial compliance increases after moderate-intensity cycling. *Am J Physiol*. 273:H2186-H2191, 1997.

61. Landmesser, U., B. Hornig, and H. Drexler. Endothelial function: A critical determinant of atherosclerosis. *Circulation*. 109:II-27 - II-33, 2004.

62. Larsen, A. I. Effect of aerobic exercise training on plasma levels of tumor necrosis factor alpha in patients with heart failure. *Am J Cardiol*. 88:805-808, 2001.

63. Laufs, U., S. Wassmann, T. Czech, T. Munzel, M. Eisenhauer, M. Bohm, and G. Nickenig. Physical Inactivity Increases Oxidative Stress, Endothelial Dysfunction, and Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 25:809-814, 2005.

64. Lee, I. M., H. D. Sesso, Y. Oguma, and R. S. Paffenbarger. Relative intensity of physical activity and reisk of coronary heart disease. *Circulation*. 107:1110-1116, 2003.

65. Lusis, A. Atherosclerosis. *Nature*. 407:233-241, 2000.

66. Mackinnon, L. T. *Advances in exercise immunology*. Queensland: Human Kinetics, 1999, 362.

67. Maeda, S., T. Tanabe, T. Miyauchi, T. Otsuki, J. sugawara, M. Iemitsu, S. Kuno, R. Ajisaka, I. Yamaguchi, and M. Matsuda. Aerobic exercise training reduces plasma endothelin-1 concentration in older women. *J Appl Physiol*. 95:336-341, 2003.

68. Maiorana, A., F. O'Droscoll, R. Taylor, and D. Green. Exercise and the Nitric Oxide Vasodilator System. *Sports Med*. 33:1013-1035, 2003.

69. Martinsen, E. W. Benefits of exercise for the treatment of depression. *Sports Medicine*. 9:380-389, 1990.

70. Masaki, T. The discovery, the present state, and the future prospects of endothelin. *J Cardiovasc Pharmacol*. 13:S1-S4, 1989.

71. Matata, B. and M. Galinanes. Effect of diabetes on nitric oxide metabolism during cardiac surgery. *Diabetes*. 50:2603-2611, 2001.

72. Meldrum, D. R. Tumor necrosis factor in the heart. *Am J Physiol*. 274:R577-R595, 1998.

73. Minetto, M., A. Rainoldi, M. Gazzoni, M. Terzolo, P. Borrione, A. Termine, L. Saba, A. Dovio, A. Angeli, and P. Paccotti. Differential responses of serum and salivary interleukin-6 to acute strenuous exercise. *European Journal of Applied Physiology*. 93:679-686, 2005.

74. Moe, I. T., H. Hoven, E. V. Hetland, O. Rognmo, and S. A. Slordahl. Endothelial function in highly endurance-trained and sedentary healthy women. *Vasc Med*. 10:97-102, 2005.

75. Moldoveanu, A., R. J. Shephard, and P. N. Shek. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol*. 89:1499-1504, 2000.

76. Moncada, S. Biological importance of prostacyclin. *Br J Pharmacol*. 76:3-31, 1982.

77. Moncada, S. and A. Higgs. Mechanims of disease: the L-Arginine-Nitric Oxide pathway. *The New England Journal of Medicine*. 329:2002-2012, 1993.

78. Moriguchi, J., H. Itoh, S. Harada, K. Takeda, T. Hatta, T. Nakata, and S. Sasaki. Low frequency regular exercise imporves flow mediated dilation of subjects with mild hypertension. *Hypertension Res*. 28:315-321, 2005.

79. Munro, J. M. and R. S. Cotran. The pathogenesis of atherosclerosis: atherogenesis and inflammation. *Lab Invest*. 58:249-261, 1988.

80. Natelson, B. H., X. Zhou, J. E. Ottenweller, M. T. Bergen, S. A. Sisto, S. Drastal, W. N. Tapp, and W. L. Gause. Effect of acute exhausting exercise on cytokine gene expression in men. *Int J Sports Med*. 17:299-302, 1996.

81. Nichols, D. and T. Glenn. Effects of aerobic exercise on pain perception, affect, and level of disability in individuals with fibromyalgia. *Phys. Ther.* 74:327-332, 1994.

82. Niebauer, J. and J. P. Cooke. Cardiovascular effects of exercise: Role of endothelial shear stress. *J Am Coll Cardiol*. 28:1652-1660, 1996.

83. Nieman, D. C., S. L. Nehlsen-Cannarella, O. R. Fagoaga, D. A. Hanson, A. Utter, J. M. Davis, F. Williams, and D. E. Butterworth. Influence of mode and

carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc*. 30:671-678, 1998.

84. O'Sullivan, S. E. The effects of exercise training on markers of endothelial function in healthy men. *Int J Sports Med*. 24:404-409, 2003.

85. Palmer, R. M., A. G. Ferrige, and S. Moncada. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 327:524-526, 1987.

86. Parise, G., S. M. Phillips, J. J. Kaczor, and Tarnopolsky. Antioxidant enzyme activity is up-regulated after unilateral resistance exercise training in older adults. *Free Rad Bio Med*. 39:289-295, 2005.

87. Parnell, M., D. P. Holst, and D. M. Kaye. Augmentation of endothelial function follwoing exercise training is associated with increased L-arginine transport in human heart failure. *Clin Sci*. 109:523-530, 2005.

88. Pedersen, B. K., A. Steensberg, C. Fischer, C. Keller, P. Keller, P. Plomgaard, E. Wolsk-Peterson, and M. Febbraio. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Preceedings of the Nutrition Society*. 63:263-267, 2004.

89. Pedersen, B. K., A. Steensberg, and P. Schjerling. Muscle-derived interleukin-6: possible biological effects. *J Physiol*. 536:329-337, 2001.

90. Pulse_Trace. Available at: www.pulsetrace.com Accessed.

91. Pyke, K. E. and M. E. Tschakovsky. The relationship between shear stress and flow-mediated dilation: implications for the assessment of endothelial function. *J Physiol*. 568:327-369, 2005.

92. Ross, R. Atherosclerosis - An inflammatory disease. *N Engl J Med*. 340:115-126, 1999.

93. Ross, R. The pathogenesis of atherosclerosis-an update. *N Engl J Med*. 314:488-500, 1986.

94. Rubanyi, G. M. The role of endothelium in cardiovascular homeostasis and disease. *Journal of Cardiovascular Pharmacology*. 22:S1-S14, 1993.

95. Rubanyi, G. M. The role of the endothelium in cardiovascular homeostasis and disease. *J Cardiovasc Pharmacol*. 22:S1-S14, 1993.

96. Rush, J. W. E., J. R. Turk, and M. H. Laughlin. Exercise training regulated SOD-1 and oxidative stress in porcine aortic endothelium. *Am J Physiol Heart Circ Physiol*. 284:H1378-H1387, 2002.

97. Rywik, T., M. Blackman, A. Yataco, P. Vaitkevicius, R. Zink, E. Cottrell, J. Wright, L. Katzel, and J. Fleg. Enhanced endothelial vasoreactivity in endurance trained older men. *J Appl Physiology*. 87:2136-2142, 1999.

98. Seals, D. R. Habitual exercise and the age-associated decline in large artery compliance. *Exerc Sport Sci Rev*. 31:68-72, 2003.

99. Sessa, W. C. eNOS at a glance. *J Cell Sci*. 117:2427-2429, 2004.

100. Shephard, R. J. Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. *Crit Rev Immunol*. 22:165-182, 2002.

101. Shephard, R. J. Sepsis and mechanisms of inflammatory response: Is exercise a good model. *Br J Sports Med*. 35:223-230, 2001.

102. Shin, Y. H., H. K. Hur, N. J. Pender, H. J. Jang, and M. S. Kim. Exercise self-efficacy, exercise benefits and barriers, and commitment to a plan for exercise among korean women with osteoporosis and osteoarthritis. *Int J Nurs. Stud*. 43:3-10, 2006.

103. Silvestro, A., F. Scopacasa, G. Oliva, T. d. Cristofaro, L. Iuliano, and G. Brevetti. Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. *Atherosclerosis*. 165:277-283, 2002.

104. Spence, A. *Basic Human Anatomy*. 3 ed. Redwood City: The Benjamin/Cummings Publishing Company, Inc, 1990, 303.

105. Starkie, R., S. R. Ostrowski, S. Jauffred, M. Febbraio, and B. K. Pedersen. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha; production in humans. *FASEB J.*:02-0670fje, 2003.

106. Starkie, R. L., J. Rolland, D. J. Angus, M. J. Anderson, and M. A. Febrraio. Circulating moncytes are not the source of elvations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Cell Physiol*. 280:C769-C774, 2001.

107. Steensberg, A., C. Keller, R. L. Starkie, T. Osada, M. A. Febbraio, and B. K. Pedersen. IL-6 and TNF-a expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab*. 283:E1272-E1278, 2002.

108. Taddei, S., F. Galetta, A. Virdis, L. Ghiadoni, G. Salvetti, F. Franzoni, C. Giusti, and A. Salvetti. Physical activity prevents age related impairment in nitric oxide availability in elderly athletes. *circulation*. 101:2896-2901, 2000.

109. Takase, B., A. Uehata, T. Akima, T. Nagai, T. Nishioka, A. Hamabe, K. Satomura, F. Ohsuzu, and A. Kurita. Endothelium-dependent flow-mediated

vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol*. 82:1535-1539, 1998.

110. Tanaka, H., F. A. Dinenno, K. D. Monahan, C. M. Clevenger, C. A. DeSouza, and D. R. Seals. Aging, Habitual Exercise, and Dynamic Arterial Compliance. *Circulation*. 102:1270-1275, 2000.

111. Tanasescu, M., M. F. Leitzmann, E. B. Rimm, W. C. Willett, M. J. Stampfer, and F. B. hu. Exercise type and intensity in relation to coronary heart disease in men. *JAMA*. 288:1994-2000, 2002.

112. Thompson, P. D., S. F. Crouse, B. Goodpaster, D. Kelley, N. Moyna, and L. Pescatello. The acute versus chronic response to exercise. *Med Sci Sports Exerc*. 33:S438-S445, 2001.

113. Tozzi-ciancarelli, M. G., M. Penco, and C. D. MAssimo. Influence of acute exercise on human platelet responsiveness: possible involvement of exercise-induced oxidative stress. *Eur J App Physiol*. 86:266-272, 2002.

114. Tulppo, N. P., A. J. Hautala, R. H. Makikallio, R. T. Laukkanen, S. Nissila, R. L. Hughson, and H. V. Huikuri. Effects of aerobic training on hearth rate dynamics in sedentary subjects. *J Appl Physiol*. 95:364-372, 2003.

115. Uehata, A., E. H. Lieberman, M. D. Gerhard, T. J. Anderson, P. Ganz, J. F. Polak, M. A. Creager, and A. C. Yeung. Noninvasive assessment fo endothelium-dependent flow-mediated dilation of the brachial artery. *Vascular Medicine*. 2:87-92, 1997.

116. Ullum, H., P. M. Haahr, M. Diamant, J. Palmo, J. Halkjaer-Kristensen, and B. K. Pedersen. Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha,

IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC. *J Appl Physiol*. 77:93-97, 1994.

117. US_Department_of_Health_and_Human_Services. *A report of the Surgeon General*, 1996

118. Vaitkevicius, P. V., J. L. Fleg, J. H. Engel, F. C. O'Connor, J. G. Wright, L. E. Lakatta, C. P. Frank, and E. G. Lakatta. Cardiovascular risk factors, lipids, and insulin: Effects of age and aerobic capacity on arterial stiffness in health adults. *Circulation*. 88:1456-1462, 1993.

119. Vane, J. R., E. E. Anggard, and R. M. Botting. Regulatory functions of the vascular enodothelium. *The New England Journal of Medicine*. 323:27-36, 1990.

120. Verma, S., M. R. Buchanan, and T. J. Anderson. Endothelial function testing as a biomarker of vascular disease *Circulation*. 108:2054-2059, 2003.

121. Vincent, H. K., J. W. Morgan, and K. R. Vincent. Obesity exacerbates oxidative stress levels after acute exercise. *Med Sci Sports Exerc*. 36:772-779, 2004.

122. Vona, M., A. Rossi, P. Capodaglio, S. Rizzo, P. Servi, M. DeMarchi, and F. Cobelli. Impact of physical training and detraining on endothelium-dependent vasodilation in patients with recent acute myocardial infarction. *Am Heart J*. 147:1039-1046, 2004.

123. Walther, C., S. Gielen, and R. Hambrecht. The effect of exercise training on endothelial function in cardiovascular disease in humans. *Exercise and Sport Sciences Reviews*. 32:129-134, 2004.

124. Wang, J., M. S. Wolin, and T. H. Hintze. Chronic exercise enhances endothelium-mediated dilation fo epicardial coronary artery in conscious dogs. *Circ Res*. 73:829-838, 1993.

125. Watts, K., P. Beye, A. Siafarikas, E. A. Davis, T. W. Jones, G. O'Driscoll, and D. J. Green. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. *J. Am. Coll. Cardiol.* 43:1823-1827, 2004.

126. Willer, A. Cancer risk reduction by physical exercise. *World Rev of Nutr and Dietetics*. 94:176-188, 2005.

127. Zhang, X., S. P. Zhao, X. P. Li, M. Gao, and Q. C. Zhou. Endothelial-dependent and -independent functions are impared in patients with coronary heart disease. Atherosclerosis. 149:19-24, 2000.

APPENDIX B – RAW DATA TABLES

TABLE 1. SUBJECT DEMOGRAPHICS

Subject #	Sex	Age (yrs)	Height (cm)	Weight (kg)	BMI (ht2/kg)	Exercise/Week	Resting SBP	Resting DBP	Fasting Glucose	TG
3042	M	62	175.2	81.93	26.7	1.5	107	79	89	51
3043	M	46	177.6	96.75	30.7	0.0	110	72	95	246
3044	M	58	166.2	80.15	29.0	1.5	122	70	97	137
3045	M	63	181.9	102.6	31.0	0.0	122	89	96	98
3046	M	53	177.2	87.1	27.7	1.5	116	73	92	182
3047	M	50	171	101.8	34.8	1.0	116	90	103	289
3050	M	68	171.4	84.31	28.7	0.0	132	82	90	176
3054	M	55	174.4	77.16	25.4	0.0	120	80	98	155
Mean		56.9	174.4	89.0	29.3	0.7	118.1	79.4	95.0	166.8
SD		7.3	4.8	10.0	2.9	0.8	7.8	7.5	4.6	76.2
SEM		2.6	1.7	3.5	1.0	0.3	2.8	2.7	1.6	27.0
3048	M	66	181	89	27.2	3.0	110	60	87	151
3049	M	47	179.5	101.4	31.5	4.0	106	82	94	74
3051	M	65	173.6	79.5	26.4	5.0	122	78	77	74
3052	M	68	173.7	75.5	25.0	7.0	102	76	96	176
3053	M	62	174.6	86.13	28.3	3.5	96	70	95	109
3055	M	54	173.1	81.55	27.2	4.0	126	72	92	114
3056	M	66	180.7	87.13	26.7	3.5	132	76	89	39
3057	M	51	174.2	81.81	27.0	5.0	126	72	109	130
Mean		59.9	176.3	85.3	27.4	4.4	115.0	73.3	92.4	108.4
SD		8.0	3.4	7.9	1.9	1.3	13.2	6.6	9.1	44.8
SEM		2.8	1.2	2.8	0.7	0.5	4.7	2.3	3.2	15.8

BMI – Body mass index, SBP – Systolic blood pressure (mm Hg), DBP – Diastolic blood pressure (mm Hg),
TG – Triglycerides (mg dL)

TABLE 1. (Continued) SUBJECT DEMOGRAPHICS

Subject #	CRP	TC	LDL	HDL	VO2peak (ml/kg)
3042	0.62	141.0	78.0	53.0	31.6
3043	1.58	218.0	135.0	34.0	31.4
3044	6.31	148.0	77.0	44.0	36.8
3045	18.3	218.0	116.0	82.0	31.5
3046	0.84	184.0	96.0	52.0	31.3
3047	2.55	204.0	111.0	35.0	27.5
3050	1.01	204.0	130.0	39.0	25.2
3054	0.54	138.0	62.0	45.0	32.0
Mean	3.97	181.9	100.6	48.0	30.9
SD	6.10	34.5	26.7	15.4	3.4
SEM	2.16	12.2	9.4	5.5	1.2
3048	2.89	242.0	156.0	56.0	40.1
3049	1.01	218.0	135.0	68.0	35.1
3051	0.61	193.0	119.0	59.0	36.2
3052	0.52	185.0	112.0	38.0	25.4
3053	1.06	158.0	107.0	29.0	36.0
3055	0.91	275.0	202.0	50.0	38.1
3056	2.42	148.0	80.0	60.0	33.4
3057	2.30	222.0	122.0	74.0	29.6
Mean	1.47	205.1	129.1	54.3	34.2
SD	0.92	42.6	36.7	14.9	4.7
SEM	0.33	15.1	13.0	5.3	1.7

CRP – C-reactive protein (mg/L), TC – Total cholesterol (mg/dL), LDL – Low-density lipoprotein cholesterol (mg/dL), HDL – High-density lipoprotein (mg/dL)

TABLE 2. EXERCISE INDUCED VARIABLES

Subject #	VO2 (25%)	RPE (25%)	HR (25%)	SBP(25%)	DBP(25%)	VO2 (50%)	RPE (50%)	HR(50%)	SBP(50%)	DBP(50%)
3042	8.4	10	104	104	66	16.2	12.0	141	138	70
3043	7.76	8.5	95	108	69	16.0	14.6	123	122	68
3044	9.33	7	72	119	77	18.9	10.0	92	133	67
3045	7.79	9	83	127	76	16.2	9.5	100	160	82
3046	8.2	7	86	128	87	15.5	11.0	116	137	76
3047	7.3	7.25	97	138	83	13.3	10.4	117	172	82
3050	7.34	7.9	81	141	101	12.2	12.0	106	146	89
3054	8.1	7.6	84	117	70	16.5	11.0	116	131	62
Mean	8.0	8.0	87.8	122.8	78.4	15.6	11.3	113.8	142.4	74.5
SD	0.7	1.1	10.0	13.2	11.6	2.1	1.6	14.9	16.4	9.3
SEM	0.2	0.4	3.5	4.7	4.1	0.7	0.6	5.3	5.8	3.3
3048	10.19	8	59	99	63	20.6	13.0	89	130	73
3049	9.26	7.6	80	133	94	17.8	11.8	118	148	77
3051	9.2	8.1	74	122	78	18.4	11.9	104	135	75
3052	7.5	7	74	105	63	12.8	9.0	100	114	69
3053	8.7	8.25	84	145	73	17.9	11.5	111	114	62
3055	9.39	7.6	77	119	65	18.6	11.9	102	132	63
3056	8.47	8	72	141	75	16.5	13.0	104	160	70
3057	7.09	7	84	144	82	15.4	9.0	115	124	75
Mean	8.7	7.7	75.5	125.8	74.1	17.3	11.4	105.4	132.1	70.4
SD	1.0	0.5	8.2	17.8	10.9	2.4	1.6	9.2	15.9	5.7
SEM	0.4	0.2	2.9	6.3	3.8	0.8	0.6	3.2	5.6	2.0

RPE – Rating of perceived exertion (6-20), HR – Heart rate, Systolic blood pressure (mm Hg)
DBP – Diastolic blood pressure (mm Hg)

TABLE 2. (Continued) EXERCISE INDUCED VARIABLES

Subject #	RPE (50%R)	HR(50%R)	SBP(50%R)	DBP(50%R)	VO2 (75%)	RPE (75%)	HR(75%)	SBP(75%)	DBP(75%)
3042	12.0	137	137	79.3	23.5	14.0	162	140	68
3043	14.7	108.1	129	69.2	23.5	17.2	150	159	79
3044	11.5	97.4	134.8	72	28.7	14.0	126	159	70
3045	9.0	86.9	139.6	78.2	22.9	13.2	140	190	80
3046	11.0	116.6	137.6	78.9	24.7	13.9	146	154	76
3047	10.1	114.6	144	76.8	19.6	14.3	145	196	79
3050					18.4	12.8	126	164	94
3054					25.1	14.0	150	155	55
Mean	11.4	110.1	137.0	75.7	23.3	14.2	143.3	164.4	75.1
SD	1.9	17.3	5.0	4.2	3.2	1.3	12.4	19.1	11.3
SEM	0.7	6.1	1.8	1.5	1.1	0.5	4.4	6.8	4.0
3048	12.6	83.8	130.5	70	30.5	14.5	132	157	65
3049					26.9	14.9	161	180	79
3051					27.9	12.9	127	156	78
3052					19.6	11.5	133	114	58
3053	10.4	116.1	112.3	63.5	26.9	15.3	140	147	62
3055	12.0	104.1	135.5	54.3	28.5	13.8	141	166	64
3056					24.6	13.8	130	191	76
3057					21.5	13.1	151	150	77
Mean	11.7	101.3	126.1	62.6	25.8	13.7	139.2	157.5	69.6
SD	1.1	16.3	12.2	7.9	3.7	1.2	11.6	23.1	8.4
SEM	0.4	5.8	4.3	2.8	1.3	0.4	4.1	8.2	3.0

RPE – Rating of perceived exertion (6-20), HR – Heart rate, Systolic blood pressure (mm Hg), R – reproducibility, DBP – Diastolic blood pressure (mm Hg)

TABLE 3. FLOW-MEDIATED DILATION SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	14.39219	14.28968	11.0932	12.41165	9.883886
R3043	6.411502	3.434304	9.192037	4.188895	10.82431
R3044	8.426184	2.422548	2.783019	5.299861	6.413302
R3045	1.778291	4.220009	4.295333	4.507435	5.91716
R3046	6.689965	4.891304	6.364562	6.18478	4.572009
R3047	12.75964	7.089552	9.706601	8.940397	8.606752
R3048	3.355057	4.883382	1.319003	-1.26614	1.15636
R3049	6.939281	11.3477	10.28278	7.42512	6.108026
R3050	9.001866	7.465155	5.782629	7.157846	7.949791
R3051	6.141975	3.939674	10.04507	6.414849	10.61169
R3052	2.771809	1.626751	8.188825	0.877983	4.064097
R3053	1.756919	4.366919	6.109635	3.597296	4.584248
R3054	7.025219	7.746281	5.443819	6.336854	8.504484
R3055	6.623979	3.472683	7.870882	9.347874	4.993283
R3056	4.253713	0.721696	4.795521	1.540313	2.958153
R3057	9.434792	12.0399	16.69184	15.8793	11.17346
Mean	6.735149	5.872346	7.497798	6.177769	6.770063
SD	3.591203	3.874962	3.727418	4.261778	3.005329
SEE	0.897801	0.968741	0.931855	1.065444	0.751332
N=	16	16	16	16	16

FMD is expressed as a percent (%)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 4. FLOW-MEDIATED DILATION SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	6.19252	8.593272	6.631709	9.798832	8.930292
R3043	8.658281	8.163265	4.279919	10.53719	2.588528
R3044	14.11146	6.898215	5.633479	1.114801	4.42055
R3045	4.716336	1.644885	4.449541	4.498594	3.69357
R3046	7.55988	2.39899	3.096903	4.802831	6.678608
R3047	8.565154	5.377658	7.647345	9.846079	7.573599
R3048	4.252704	1.079963	4.186834	2.480774	3.110571
R3049	12.5194	17.64072	17.08323	9.888399	8.869239
R3050	3.397028	5.258216	5.521749	4.192726	5.081157
R3051	6.590347	7.210626	6.037152	6.523116	8.333333
R3052	4.067642	1.110854	5.860983	3.60549	5.531506
R3053	6.600249	5.589744	8.192231	3.559707	4.138106
R3054	8.600159	4.904338	6.893777	9.6	8.121411
R3055	9.250903	5.147392	4.808331	6.450878	4.151706
R3056	1.389227	-3.97045	2.389967	-0.21388	4.777308
R3057	10.5549	6.764082	13.1712	10.97473	11.90818
Mean	7.314137	5.238236	6.617772	6.103767	6.119229
SD	3.386625	4.640193	3.730859	3.6189	2.602852
SEE	0.846656	1.160048	0.932715	0.904725	0.650713
N=	16	16	16	16	16

FMD is expressed as a percent (%)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 5. FLOW-MEDIATED DILATION SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	3.896104	2.118138	4.172921	5.0625	9.042049
R3043	8.201058	6.604375	8.571985	7.716049	7.610063
R3044	7.652823	4.902416	5.097947	5.260594	5.288578
R3045	7.729575	5.598622	4.831338	7.667659	8.514851
R3046	13.23496	5.715552	7.627866	8.623771	6.59293
R3047	6.035122	3.059478	3.29849	9.124907	7.070949
R3048	2.696133	-1.12474	3.359766	2.486002	0.563063
R3049	12.84523	11.19247	13.68204	9.851952	8.2054
R3050	8.55792	3.773585	4.903955	6.378132	2.95104
R3051	6.244057	7.160883	10.94205	8.759362	8.383035
R3052	3.219829	-3.76263	4.988345	4.100069	6.213365
R3053	6.560738	2.784388	8.571429	4.856568	1.276596
R3054	8.0466	6.073753	7.807571	5.772314	6.522883
R3055	5.723669	4.072598	9.090909	1.79301	2.79476
R3056	7.476869	0.996755	1.222379	2.455032	3.708222
R3057	10.17935	6.857826	8.987952	11.26695	8.241506
Mean	7.393752	4.126466	6.697309	6.323429	5.811205
SD	2.973118	3.545246	3.246753	2.828959	2.734763
SEE	0.743279	0.886311	0.811688	0.70724	0.683691
N=	16	16	16	16	16

FMD is expressed as a percent (%)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 6. FLOW-MEDIATED DILATION SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	4.220293	6.251973	5.649718	3.625838	8.131068
R3043	8.02626	7.591457	5.122951	14.87475	6.724283
R3044	7.05913	9.036922	5.238221	2.576408	6.525038
R3045	5.336154	1.755986	4.62963	2.801978	1.950313
R3046	9.951764	4.397852	6.997389	7.82331	5.177665
R3047	11.94409	10.16097	10.7089	5.886736	5.643013
R3048	3.549161	0.479343	1.824292	2.177636	3.311753
R3049					
R3050					
R3051					
R3052					
R3053	5.78215	4.112923	6.476868	6.202501	6.656841
R3054					
R3055	6.891767	4.303233	6.866953	5.162175	4.035167
R3056					
R3057					
Mean	6.973419	5.343406	5.946102	5.681259	5.350571
SD	2.695033	3.21615	2.369284	3.938948	1.945129
SEE	0.673758	0.804038	0.592321	0.984737	0.486282
N=	16	16	16	16	16

FMD is expressed as a percent (%)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 7. BASELINE DIAMETER SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	3.891	3.604	3.723	3.537	3.531
R3043	5.147	5.183	5.124	5.061	4.804
R3044	4.308	4.293	4.24	4.302	4.21
R3045	4.33	4.218	4.307	4.304	4.056
R3046	4.006	4.048	3.928	3.929	3.937
R3047	4.044	4.02	4.09	4.228	4.206
R3048	4.143	4.116	4.094	4.028	3.978
R3049	4.035	3.851	3.89	3.973	3.962
R3050	4.288	4.233	4.306	4.289	4.302
R3051	3.24	3.249	3.106	3.071	2.959
R3052	4.654	4.426	4.152	4.442	4.306
R3053	4.155	4.099	4.141	4.142	4.101
R3054	3.886	3.563	3.729	3.598	3.457
R3055	4.529	4.521	4.523	4.493	4.466
R3056	3.973	4.434	4.108	4.155	4.158
R3057	4.547	4.211	3.972	4.043	4.099
Mean	4.1985	4.129313	4.089563	4.099688	4.03325
SD	0.41869	0.525621	0.484344	0.504384	0.416596

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 8. BASELINE DIAMETER SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	3.262	3.27	3.242	3.082	3.113
R3043	4.77	4.802	4.93	4.84	4.829
R3044	4.181	4.146	4.349	4.216	4.185
R3045	4.389	4.438	4.36	4.268	4.386
R3046	3.939	3.911	3.83	3.758	3.94
R3047	4.098	4.091	4.106	4.093	4.212
R3048	4.068	4.352	4.132	4.031	4.115
R3049	3.866	3.713	3.881	3.853	3.732
R3050	4.239	4.26	4.437	4.317	4.251
R3051	3.232	3.162	3.23	3.158	3.108
R3052	4.376	4.321	4.129	4.299	4.158
R3053	4.015	3.9	4.016	3.961	3.939
R3054	3.779	3.711	3.728	3.625	3.657
R3055	4.432	4.41	4.513	4.387	4.456
R3056	4.103	4.332	4.226	4.208	4.019
R3057	4.235	4.243	4.153	4.155	4.182
Mean	4.0615	4.066375	4.078875	4.015688	4.017625
SD	0.503368	0.516392	0.569167	0.587028	0.571327

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 9. BASELINE DIAMETER SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	3.234	3.352	3.331	3.2	3.163
R3043	3.78	4.709	5.133	4.86	4.77
R3044	4.286	4.304	4.237	4.106	4.141
R3045	4.541	4.644	4.595	4.369	4.545
R3046	4.337	4.514	4.536	4.476	4.611
R3047	4.043	4.674	4.305	4.011	4.172
R3048	4.525	4.89	4.792	4.465	4.44
R3049	3.838	3.824	3.881	3.715	3.778
R3050	4.23	4.399	4.425	4.39	4.473
R3051	3.155	3.17	3.089	3.071	3.018
R3052	4.317	4.651	4.29	4.317	4.265
R3053	3.902	4.202	3.955	3.974	3.995
R3054	3.691	3.688	3.804	3.742	3.802
R3055	4.263	4.518	4.323	4.406	4.58
R3056	3.999	4.314	4.254	4.114	4.099
R3057	4.126	4.185	4.15	4.278	4.356
Mean	4.016688	4.252375	4.19375	4.093375	4.138
SD	0.472633	0.518409	0.593403	0.56207	0.580634

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 10. BASELINE DIAMETER SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	3.341	3.167	3.363	3.282	3.296
R3043	4.722	4.729	4.88	4.511	4.744
R3044	3.839	3.873	3.799	3.959	3.954
R3045	4.254	4.385	4.32	4.247	4.307
R3046	4.008	3.96	4.004	3.956	3.908
R3047	3.935	3.976	3.978	4.026	4.129
R3048	4.17	4.381	4.166	4.087	4.016
R3049					
R3050					
R3051					
R3052					
R3053	4.168	4.109	4.215	4.079	4.071
R3054					
R3055	4.324	4.485	4.427	4.378	4.436
R3056					
R3057					
Mean	4.084556	4.118333	4.128	4.058333	4.095667
SD	0.378821	0.455261	0.423102	0.347853	0.400569

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 11. HYPEREMIC DIAMETER SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	4.451	4.119	4.136	3.976	3.88
R3043	5.477	5.361	5.595	5.273	5.324
R3044	4.671	4.397	4.358	4.53	4.48
R3045	4.407	4.396	4.492	4.498	4.296
R3046	4.274	4.246	4.178	4.172	4.117
R3047	4.56	4.305	4.487	4.606	4.568
R3048	4.282	4.317	4.148	3.977	4.024
R3049	4.315	4.288	4.29	4.268	4.204
R3050	4.674	4.549	4.555	4.596	4.644
R3051	3.439	3.377	3.418	3.268	3.273
R3052	4.783	4.498	4.492	4.481	4.481
R3053	4.228	4.278	4.394	4.291	4.289
R3054	4.159	3.839	3.932	3.826	3.751
R3055	4.829	4.678	4.879	4.913	4.689
R3056	4.142	4.466	4.305	4.219	4.281
R3057	4.976	4.718	4.635	4.685	4.557
Mean	4.479188	4.3645	4.393375	4.348688	4.303625
SD	0.431717	0.448394	0.537683	0.44527	0.497594

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 12. HYPEREMIC DIAMETER SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	3.464	3.551	3.457	3.384	3.391
R3043	5.183	5.194	5.141	5.35	4.954
R3044	4.771	4.432	4.594	4.263	4.37
R3045	4.596	4.511	4.554	4.46	4.548
R3046	4.331	4.083	4.098	4.052	4.144
R3047	4.449	4.311	4.42	4.496	4.531
R3048	4.241	4.399	4.305	4.131	4.243
R3049	4.35	4.368	4.544	4.234	4.063
R3050	4.383	4.484	4.682	4.498	4.467
R3051	3.445	3.39	3.425	3.364	3.367
R3052	4.554	4.369	4.371	4.454	4.388
R3053	4.28	4.118	4.345	4.102	4.102
R3054	4.104	3.893	3.985	3.973	3.954
R3055	4.842	4.637	4.73	4.67	4.641
R3056	4.16	4.16	4.327	4.199	4.211
R3057	4.682	4.53	4.7	4.611	4.68
Mean	4.364688	4.276875	4.354875	4.265063	4.253375
SD	0.573852	0.539655	0.563596	0.642097	0.52819

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 13. HYPEREMIC DIAMETER SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	3.36	3.423	3.47	3.362	3.449
R3043	4.09	5.02	5.573	5.235	5.133
R3044	4.614	4.515	4.453	4.322	4.36
R3045	4.892	4.904	4.817	4.704	4.932
R3046	4.911	4.772	4.882	4.862	4.915
R3047	4.287	4.817	4.447	4.377	4.467
R3048	4.647	4.835	4.953	4.576	4.465
R3049	4.331	4.252	4.412	4.081	4.088
R3050	4.592	4.565	4.642	4.67	4.605
R3051	3.352	3.397	3.427	3.34	3.271
R3052	4.456	4.476	4.504	4.494	4.53
R3053	4.158	4.319	4.294	4.167	4.046
R3054	3.988	3.912	4.101	3.958	4.05
R3055	4.507	4.702	4.716	4.485	4.708
R3056	4.298	4.357	4.306	4.215	4.251
R3057	4.546	4.472	4.523	4.76	4.715
Mean	4.314313	4.421125	4.47	4.3505	4.374063
SD	0.58811	0.588955	0.692093	0.640557	0.612194

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 14. HYPEREMIC DIAMETER SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	3.482	3.365	3.553	3.401	3.564
R3043	5.101	5.088	5.13	5.182	5.063
R3044	4.11	4.223	3.998	4.061	4.212
R3045	4.481	4.462	4.52	4.366	4.391
R3046	4.311	4.055	4.128	4.146	4.169
R3047	4.405	4.38	4.404	4.263	4.362
R3048	4.318	4.402	4.242	4.176	4.149
R3049					
R3050					
R3051					
R3052					
R3053	4.409	4.278	4.488	4.332	4.342
R3054					
R3055	4.622	4.678	4.731	4.604	4.615
R3056					
R3057					
Mean	4.359889	4.325667	4.354889	4.281222	4.318556
SD	0.429293	0.466585	0.450592	0.471478	0.400248

Diameter is expressed in millimeters (mm)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 15. BASELINE VELOCITY SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	16	19.1	21.7	15.2	16.5
R3043	37	41.8	35.8	22.4	13.2
R3044	16	24	13.4	12.8	10.6
R3045	13	27.2	12.9	9.7	7.3
R3046	20	23	11.4	13.2	9.2
R3047	12	15.5	7.3	9.3	11.1
R3048	24	12.6	7.3	6.3	9.9
R3049	22	20.5	15.7	7.7	7.4
R3050	36	20	19.6	19.1	15.9
R3051	12	16.5	7.9	10	8.5
R3052	11	9.6	11.3	7.6	6.9
R3053	29	10.8	10.9	6.2	7.7
R3054	18	31.3	9.5	8.2	8.6
R3055	20	21.2	22.4	19.6	12
R3056	19	34.2	22.3	11.9	11.4
R3057	12	13.7	16	11.5	11.8
Mean	19.7375	21.3125	15.3375	11.91875	10.5
SD	9.024781	9.133236	10.30251	4.78191	3.212112

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 16. BASELINE VELOCITY SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	19.6	27.5	24.9	18.8	13.7
R3043	29.7	25.9	29.5	17	11.9
R3044	20.8	29.8	23.8	16.6	13.8
R3045	18.3	25.1	13.5	16.6	14.3
R3046	18.8	23.1	12.4	10.5	8.8
R3047	10.9	23	11.9	8.9	9.6
R3048	10.2	34.2	13.8	10.1	8.6
R3049	23.6	21.1	20.2	10.7	8.2
R3050	30.8	25.5	15.9	9.8	11.8
R3051	12.9	18.4	10.7	7.4	9.1
R3052	7.4	16.4	8.9	8.7	9.1
R3053	38.9	23.7	14	9.1	8.8
R3054	15.4	27.3	15.9	12.4	11.1
R3055	21.1	14.5	14.6	12.1	9.6
R3056	21.7	27.7	13.1	12.5	12.4
R3057	12.5	19.2	11.9	13.1	12.4
Mean	19.5375	23.9	15.9375	12.14375	10.825
SD	6.023759	2.626531	7.637452	4.014806	2.34215

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 17. BASELINE VELOCITY SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	25.3	33.5	16	13.6	15.2
R3043	27.4	27.3	35.4	23.8	10.9
R3044	15.8	31.2	17.3	12.8	11.1
R3045	18.1	36.3	20.8	24.4	21.2
R3046	22.1	25.8	25	11.6	8.4
R3047	10.1	15.5	13.1	12.7	10.4
R3048	16.4	46.8	12.1	10.1	8.2
R3049	15.8	28.5	16.9	11	9.4
R3050	33.3	35.1	31.5	17	12.7
R3051	13.7	18.8	16.3	9.3	9.4
R3052	10.4	17.4	8.7	8.9	8.2
R3053	13.3	51.7	15.5	11.7	9.7
R3054	25.2	31.2	13	12.8	22.6
R3055	15	24.6	13.8	11.1	9.4
R3056	26.7	24.1	15.4	13.1	11.7
R3057	9.5	13.8	10.2	13.7	11
Mean	18.63125	28.85	17.5625	13.6	11.84375
SD	6.423083	7.355723	8.050756	5.937143	4.645715

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 18. BASELINE VELOCITY SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	36.5	36.5	28.4	14.4	22
R3043	40.2	40	20.2	29.3	13.2
R3044	20.6	24.3	7.9	8.3	13
R3045	15.2	27	14.4	13.1	8.6
R3046	12.4	23.4	9.9	11.3	8.2
R3047	12.7	20.4	14.1	10.3	11.7
R3048	14.4	34.2	9.4	10	8.3
R3049					
R3050					
R3051					
R3052					
R3053	24.9	32.6	23.5	13.1	12.5
R3054					
R3055	19.5	24.7	14.6	11.5	10
R3056					
R3057					
Mean	21.82222	29.23333	15.82222	13.47778	11.94444
SD	10.24985	6.76997	6.909012	6.216265	4.278467

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 19. HYPEREMIC VELOCITY SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	107.8	125.2	146.7	145.8	132.1
R3043	109.6	143.4	95.8	99.7	104.7
R3044	93.3	94.7	95	84.3	86.6
R3045	93.3	78	70.9	80.6	73.7
R3046	122.3	97.2	94.3	113	104.3
R3047	114.6	104.8	86	89.4	103.1
R3048	73.5	65.4	69.7	57.8	81.4
R3049	132.5	94.5	102	103.3	90.2
R3050	115	102.9	113.7	121.1	117.5
R3051	80	74.6	81.2	81.3	87.3
R3052	80.5	65.6	61.9	72.6	66.3
R3053	57.5	53.6	48.1	57.7	55
R3054	100	103.2	90.4	79.3	66.9
R3055	73.4	134.3	73.5	78	76.3
R3056	89.1	80.8	85.4	85	80.8
R3057	83.1	94	96	103.7	89.8
Mean	106.8167	107.2167	98.11667	102.1333	100.75
SD	11.61403	23.44819	25.60292	24.39112	19.74069

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 20. HYPEREMIC VELOCITY SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	116.9	104.5	131.3	136.2	84.8
R3043	78.1	96.8	115.4	90	91.8
R3044	104.8	96.4	85.9	77.4	94
R3045	91.7	88	101.3	73	84.5
R3046	120.3	109.7	114.5	86.9	89.1
R3047	95.1	97	83.2	96.5	91.3
R3048	75.3	66.6	68.3	59.9	55.7
R3049	124.1	110.6	98	93.5	85.7
R3050	111.3	87.3	104.1	84	80.5
R3051	102.4	60	74.3	73.5	83.6
R3052	57.6	70.5	52.9	65.1	59
R3053	83.5	79.4	71.3	70.4	66
R3054	51.7	79.4	99.7	95.4	74.3
R3055	100.2	67.3	134.7	82.5	60.4
R3056	67	60.3	68.1	75.6	82
R3057	104.1	100	92.7	110.2	94.2
Mean	101.15	98.73333	105.2667	93.33333	89.25
SD	16.03318	7.49551	18.67305	22.66007	3.889859

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 21. HYPEREMIC VELOCITY SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	87.7	112.6	100.7	99.5	108.8
R3043	118.4	110.9	114.4	107.6	98.5
R3044	85.7	96.9	79.5	78.7	65.6
R3045	96.5	94.4	102.1	78.9	93.3
R3046	101	116.4	117.4	107.5	63.9
R3047	96.5	84.5	98.4	74.7	73
R3048	71.2	79	62.9	73.2	45.3
R3049	126.9	104.6	114.2	107.5	88.7
R3050	111.7	105.3	125.3	98.6	85.6
R3051	91.8	91.5	90	75.68	89
R3052	78.5	83.8	59.8	57	52.1
R3053	84.9	80.4	73.2	70	70.1
R3054	93.6	84.1	88	83.1	70.1
R3055	90.9	69	62.8	60	66.8
R3056	117	77.3	78.9	73.6	83.2
R3057	130.6	82.3	86.1	101.5	96.6
Mean	97.63333	102.6167	102.0833	91.15	83.85
SD	11.71523	12.54327	13.50399	15.38386	18.84258

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 22. HYPEREMIC VELOCITY SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	115.4	104.4	112.4	104.5	125.4
R3043	97.8	97.8	101.7	86.2	102.7
R3044	90.1	78.1	79.7	90.4	74.4
R3045	105.1	97.1	78.8	90.8	83.9
R3046	102.1	94.9	94.9	96.8	109.8
R3047	99.8	113.2	98.6	85	99.8
R3048	92.7	66	63	57.9	66.2
R3049					
R3050					
R3051					
R3052					
R3053	58	81.9	72.3	63.2	66.4
R3054					
R3055	83.2	80.8	85.2	84.6	62.2
R3056					
R3057					
Mean	93.8	90.46667	87.4	84.37778	87.86667
SD	16.29432	14.7765	15.70287	14.95851	22.4677

Velocity is expressed in centimeters per second (cm/s)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 23. BASELINE LOCAL SHEAR STRESS SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	1.151375	1.483907	1.632017	1.20328	1.308411
R3043	1.996503	2.258152	1.956284	1.239281	0.769359
R3044	1.046425	1.565339	0.884906	0.833101	0.704988
R3045	0.860046	1.805595	0.838635	0.631041	0.503945
R3046	1.376935	1.590909	0.812627	0.940697	0.654305
R3047	0.858556	1.079602	0.499756	0.615894	0.738944
R3048	1.594979	0.857143	0.499267	0.437934	0.696833
R3049	1.498885	1.490522	1.130077	0.542663	0.522968
R3050	2.318097	1.322939	1.274501	1.246911	1.034868
R3051	1.054321	1.421976	0.71217	0.911755	0.804326
R3052	0.649764	0.60732	0.762042	0.479063	0.448676
R3053	1.920578	0.737741	0.73702	0.419121	0.525725
R3054	1.311374	2.459725	0.713328	0.638132	0.696558
R3055	1.230294	1.312984	1.38669	1.221456	0.752351
R3056	1.353134	2.159675	1.519961	0.801925	0.767677
R3057	0.745107	0.910948	1.127895	0.796438	0.80605
Mean	1.208779	1.416046	0.972778	0.783471	0.715276
SD	0.40721	0.471822	0.475894	0.288484	0.240395

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 24. BASELINE LOCAL SHEAR STRESS SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	1.682403	2.35474	2.150524	1.707982	1.232252
R3043	1.743396	1.510204	1.675456	0.983471	0.689998
R3044	1.392968	2.012542	1.532306	1.102467	0.923297
R3045	1.167464	1.583596	0.866972	1.089035	0.912905
R3046	1.33638	1.653797	0.906527	0.782331	0.625381
R3047	0.744754	1.574187	0.811495	0.608844	0.638177
R3048	0.702065	2.200368	0.93514	0.701563	0.585176
R3049	1.70926	1.591166	1.457356	0.777576	0.61522
R3050	2.034442	1.676056	1.003381	0.635627	0.777229
R3051	1.117574	1.629349	0.927554	0.656111	0.81982
R3052	0.473492	1.062717	0.603536	0.566643	0.612795
R3053	2.712827	1.701538	0.976096	0.643272	0.625539
R3054	1.141043	2.059822	1.194206	0.957793	0.849877
R3055	1.333032	0.920635	0.905828	0.772282	0.603232
R3056	1.480868	1.790397	0.86796	0.831749	0.863896
R3057	0.826446	1.267028	0.802312	0.882792	0.830225
Mean	1.206976	1.717267	1.186687	0.897602	0.765502
SD	0.451361	0.374797	0.489053	0.343198	0.207472

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 25. BASELINE LOCAL SHEAR STRESS SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	2.190476	2.798329	1.344941	1.19	1.345558
R3043	2.02963	1.623275	1.931034	1.371193	0.639832
R3044	1.032198	2.02974	1.143262	0.872869	0.750543
R3045	1.116054	2.18863	1.267465	1.563745	1.306051
R3046	1.426793	1.600354	1.54321	0.725648	0.510085
R3047	0.699481	0.928541	0.852033	0.886562	0.697987
R3048	1.014807	2.679755	0.707012	0.633371	0.517117
R3049	1.152684	2.08682	1.219273	0.829071	0.696665
R3050	2.204255	2.234144	1.99322	1.084282	0.794992
R3051	1.215848	1.660568	1.477501	0.847932	0.872101
R3052	0.674543	1.047517	0.567832	0.577253	0.538335
R3053	0.954382	3.445026	1.097345	0.824358	0.67985
R3054	1.911677	2.368764	0.956887	0.957777	1.664387
R3055	0.985222	1.524568	0.893824	0.705402	0.574672
R3056	1.869467	1.56421	1.013634	0.89159	0.799219
R3057	0.644692	0.923297	0.688193	0.896681	0.707071
Mean	1.255251	1.864353	1.205356	0.949764	0.787427
SD	0.504299	0.617477	0.411208	0.32253	0.305335

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 26. BASELINE LOCAL SHEAR STRESS SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	3.058964	3.227029	2.364555	1.228519	1.868932
R3043	2.383736	2.368365	1.159016	1.818665	0.779089
R3044	1.502475	1.756778	0.582258	0.587017	0.920587
R3045	1.00047	1.724059	0.933333	0.863668	0.55909
R3046	0.866267	1.654545	0.692308	0.799798	0.587513
R3047	0.903685	1.43662	0.992459	0.716344	0.793412
R3048	0.966906	2.185802	0.631781	0.685099	0.578685
R3049					
R3050					
R3051					
R3052					
R3053	1.672745	2.221465	1.561091	0.89924	0.85974
R3054					
R3055	1.26272	1.542029	0.923424	0.735496	0.631199
R3056					
R3057					
Mean	1.513108	2.012966	1.093359	0.925983	0.842027
SD	0.757206	0.559589	0.56334	0.381131	0.40712

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 27. HYPEREMIC LOCAL SHEAR STRESS SUMMARY (25%)

	25%				
Subject	A	B	C	D	E
R3042	7.757389	9.72697	11.03304	11.54198	10.47522
R3043	5.962308	7.746865	5.234973	5.515906	6.102415
R3044	6.064067	6.176567	6.273585	5.48675	5.75962
R3045	6.033256	5.177809	4.609241	5.243494	5.087771
R3046	8.548178	6.72332	6.721996	8.05294	7.417831
R3047	7.934718	7.299502	5.887531	5.92053	6.863528
R3048	4.967415	4.44898	4.766976	4.017875	5.729512
R3049	9.194548	6.870943	7.341902	7.280141	6.374558
R3050	7.509328	6.80652	7.393405	7.905806	7.647606
R3051	6.91358	6.429055	7.320026	7.412569	8.260899
R3052	4.843146	4.150023	4.174374	4.576317	4.311194
R3053	3.87485	3.661381	3.252355	3.900531	3.755182
R3054	7.205353	8.11002	6.787879	6.171206	5.418571
R3055	4.537867	8.317629	4.550077	4.860895	4.783699
R3056	6.279386	5.102391	5.820837	5.728039	5.441077
R3057	5.11722	6.250297	6.767372	7.181796	6.134179
Mean	6.82186	6.475003	6.336364	6.504851	6.638255
SD	1.4915	1.645713	1.992339	2.186972	1.757127

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 28. HYPEREMIC LOCAL SHEAR STRESS SUMMARY (50%)

	50%				
Subject	A	B	C	D	E
R3042	10.03433	8.948012	11.33991	12.37378	7.627369
R3043	4.584486	5.644315	6.554158	5.206612	5.322841
R3044	7.018417	6.510371	5.530467	5.140417	6.289128
R3045	5.85008	5.55205	6.505505	4.789128	5.394437
R3046	8.551409	7.853746	8.370757	6.474721	6.33198
R3047	6.497804	6.638964	5.673648	6.601515	6.069326
R3048	5.182891	4.284926	4.628267	4.160754	3.790036
R3049	8.988101	8.340426	7.070343	6.794705	6.429796
R3050	7.351734	5.738028	6.569304	5.448228	5.302282
R3051	8.871287	5.313093	6.440867	6.516783	7.531532
R3052	3.685558	4.568387	3.587309	4.240056	3.973064
R3053	5.823163	5.700513	4.971116	4.976521	4.691546
R3054	3.830643	5.990838	7.488197	7.368828	5.688816
R3055	6.330325	4.273016	8.35719	5.265557	3.795332
R3056	4.572264	3.897507	4.512068	5.030418	5.712864
R3057	6.882645	6.599104	6.24994	7.426233	6.30703
Mean	6.926437	6.365429	6.570123	6.229847	5.875951
SD	2.126844	1.589134	2.129608	2.37808	1.292759

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 29. HYPEREMIC LOCAL SHEAR STRESS SUMMARY (75%)

	75%				
Subject	A	B	C	D	E
R3042	7.593074	9.405728	8.464725	8.70625	9.631363
R3043	8.77037	6.594181	6.240405	6.199177	5.781971
R3044	5.598693	6.303903	5.253717	5.36678	4.435644
R3045	5.950231	5.691645	6.221545	5.056535	5.747855
R3046	6.520636	7.220204	7.246914	6.724754	3.880286
R3047	6.683156	5.062045	6.400000	5.21466	4.899329
R3048	4.405746	4.523517	3.675292	4.59037	2.856757
R3049	9.257947	7.658996	8.239114	8.102288	6.573849
R3050	7.393853	6.702432	7.928588	6.288838	5.358372
R3051	8.147068	8.082019	8.15798	6.900163	8.257124
R3052	5.091499	5.044937	3.90303	3.697012	3.420399
R3053	6.09226	5.357449	5.182301	4.932058	4.913141
R3054	7.100515	6.385033	6.477392	6.218065	5.162546
R3055	5.970443	4.276228	4.067546	3.812982	4.083843
R3056	8.192048	5.017153	5.19323	5.009237	5.683337
R3057	8.862821	5.506332	5.809157	6.643291	6.209366
Mean	6.801842	6.558718	6.380272	6.055799	5.548457
SD	1.608165	1.547963	1.715273	1.573608	2.143961

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 30. HYPEREMIC LOCAL SHEAR STRESS SUMMARY (50% R)

	50%R				
Subject	A	B	C	D	E
R3042	9.671356	9.230186	9.358311	8.915296	10.65291
R3043	5.799238	5.790653	5.835246	5.350477	6.061551
R3044	6.571503	5.646269	5.874177	6.393534	5.268589
R3045	6.917724	6.200228	5.107407	5.986343	5.454377
R3046	7.132735	6.710101	6.636364	6.851365	7.86694
R3047	7.101398	7.971831	6.940171	5.911575	6.76774
R3048	6.22446	4.218215	4.234277	3.966724	4.615538
R3049					
R3050					
R3051					
R3052					
R3053	3.896353	5.58092	4.802847	4.338318	4.566937
R3054					
R3055	5.387604	5.04437	5.388751	5.41069	3.92606
R3056					
R3057					
Mean	6.522486	6.265864	6.019728	5.902702	6.131183
SD	1.564368	1.527374	1.513601	1.45628	2.081997

Local shear stress is expressed in arbitrary units (AU)
A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post
R- reproducibility

TABLE 31. INTERLEUKIN-6 (IL-6) SUMMARY

	25%					50%					75%				
Subject	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
R3042	1.84		0.86			2.39	1.81	2.44	3.85	3.14	1.62		1.81		
R3043	1.27	1.33	1.39	1.50	2.28	2.25	2.35	2.75	2.84	3.75	2.62	3.39	3.19	2.82	3.87
R3044	1.98		1.75			2.10	2.57	2.36	2.71	3.41	3.44		3.25		
R3045	3.75	2.83	3.63	3.20	4.16	2.17	3.16	3.42	7.81	7.89	2.60	3.72	4.08	2.72	2.36
R3046	0.88		0.85			0.35	1.07	0.61	1.25	2.33	0.98		1.88		
R3047	1.13	1.19	1.34	2.11	2.63	1.22	1.80	2.40	2.52	2.96	0.85	4.31	3.75	3.30	2.51
R3050	2.84		2.82			2.20	2.29	3.13	2.29	2.29	2.38		2.60		
R3054	1.20		1.07			0.82	1.27	1.48	2.10	2.73	0.74		1.96		
Mean	1.86	1.78	1.71	2.27	3.02	1.69	2.04	2.32	3.17	3.56	1.90	3.81	2.82	2.95	2.91
SD	0.99	0.91	1.00	0.86	1.00	0.78	0.69	0.90	2.01	1.82	1.00	0.47	0.88	0.31	0.83
SEE	0.35	0.32	0.35	0.30	0.35	0.27	0.24	0.32	0.71	0.64	0.35	0.16	0.31	0.11	0.29
R3048	1.43		1.36			0.95	1.14	1.09	1.03	1.60	1.43		2.88		
R3049	0.61		0.40			1.52	1.61	1.55	2.63	2.69	0.60		2.54		
R3051	0.57	0.67	0.43	1.11	1.48	0.41	0.73	0.87	1.68	3.31	0.26	0.91	1.02	2.55	4.04
R3052	0.82	0.80	0.83	0.99	1.51	0.84	0.93	1.06	1.11	1.55	0.80	1.52	1.99	2.01	2.62
R3053	1.63		1.87			1.59	2.40	3.27	4.67	15.48	3.49		5.36		
R3055	1.03		1.31			0.88	1.44	1.22	1.40	1.79	0.83		1.81		
R3056	0.96	1.03	1.09	1.14	1.29	1.16	1.51	1.59	1.56	1.61	1.43	1.78	2.05	1.55	1.42
R3057	2.56		2.78			2.77	2.96	3.51	3.16	3.38	2.03		3.33		
Mean	1.20	0.84	1.26	1.08	1.42	1.26	1.59	1.77	2.16	3.92	1.36	1.41	2.62	2.04	2.69
SD	0.66	0.18	0.79	0.08	0.12	0.72	0.75	1.03	1.25	4.73	1.03	0.45	1.31	0.50	1.31
SEE	0.23	0.06	0.28	0.03	0.04	0.25	0.26	0.36	0.44	1.67	0.36	0.16	0.46	0.18	0.46

A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

TABLE 32. TUMOR NECROSIS FACTOR-ALPHA (TNF-α) SUMMARY

	25%					50%					75%				
Subject	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
R3042	0.43		0.67			0.54	0.40	0.54	0.42	0.50	0.64		0.95		
R3043	0.92	1.49	0.83	1.53	1.50	1.16	1.29	1.22	1.26	1.11	1.68	1.38	1.46	1.39	1.33
R3044	1.00		0.86			1.13	0.76	1.04	0.85	1.00	1.21		1.30		
R3045	0.68	0.60	0.62	0.78	0.42	0.95	0.36	0.66	0.52	0.57	0.88	0.99	0.92	0.61	0.76
R3046	0.25		0.27			0.67	0.40	0.60	0.38	0.41	0.37		0.45		
R3047	0.66	0.85	0.89	0.86	0.71	1.13	0.90	1.10	1.02	0.85	0.78	1.23	0.88	1.01	1.05
R3050	1.19		1.11			1.43	1.36	1.26	1.10	1.27	1.31		1.20		
R3054	0.94		1.20			1.01	0.78	1.03	1.00	0.92	0.55		0.75		
Mean	0.76	0.98	0.81	1.06	0.88	1.00	0.78	0.93	0.82	0.83	0.93	1.20	0.99	1.00	1.05
SD	0.31	0.46	0.29	0.41	0.56	0.29	0.39	0.29	0.33	0.31	0.44	0.19	0.32	0.39	0.29
SEE	0.11	0.16	0.10	0.14	0.20	0.10	0.14	0.10	0.12	0.11	0.16	0.07	0.11	0.14	0.10
R3048	1.38		1.60			1.33	1.06	1.34	1.15	1.28	1.11		1.19		
R3049	0.60		0.60			0.96	0.95	0.83	0.73	0.75	0.58		0.76		
R3051	0.71	0.99	0.61	0.82	0.83	0.75	0.55	0.95	0.40	0.47	0.58	1.33	0.72	1.57	1.12
R3052	0.73	0.56	0.78	0.89	0.46	0.59	0.71	0.63	0.72	0.67	0.54	0.84	0.43	0.86	0.69
R3053	0.63		0.56			0.95	0.84	1.03	0.75	0.77	0.56		0.68		
R3055	0.24		0.30			0.59	0.76	0.53	0.39	0.30	0.74		0.83		
R3056	0.75	0.88	0.62	0.90	0.67	0.66	0.58	0.72	0.37	0.51	0.95	1.09	0.77	0.73	0.51
R3057	0.99		1.03			0.72	0.58	0.67	0.80	0.76	1.29		1.10		
Mean	0.75	0.81	0.76	0.87	0.65	0.82	0.75	0.84	0.66	0.69	0.79	1.08	0.81	1.05	0.77
SD	0.33	0.22	0.40	0.05	0.19	0.25	0.19	0.26	0.27	0.29	0.29	0.25	0.24	0.45	0.31
SEE	0.12	0.08	0.14	0.02	0.07	0.09	0.07	0.09	0.10	0.10	0.10	0.09	0.08	0.16	0.11

A- pre-exercise, B- immediately, C- 1hr post, D- 2hr post, E- 3 hr post

APPENDIX D – STATISTICAL SUMMARIES

TABLE 1. GROUP X INTENSITY X TIME (FMD)

```
* * * * * * A n a l y s i s   o f   V a r i a n c e -- design   1 * * *

 Tests of Significance for FMD using UNIQUE sums of squares
 Source of Variation          SS      DF        MS         F  Sig of F

 Error 1                  526.36      14     37.60
 A                           .07       1       .07       .00      .967

* * * * * * A n a l y s i s   o f   V a r i a n c e -- design   1 * * *

 Tests of Significance for FMD using UNIQUE sums of squares
 Source of Variation          SS      DF        MS         F  Sig of F

 Error 2                  279.79      28      9.99
 B                           .36       2       .18       .02      .982
 A BY B                    11.51       2      5.76       .58      .569

* * * * * * A n a l y s i s   o f   V a r i a n c e -- design   1 * * *
 Tests of Significance for FMD using UNIQUE sums of squares
 Source of Variation          SS      DF        MS         F  Sig of F

 Error 3                   70.54      14      5.04
 C                          1.06       1      1.06       .21      .654
 A BY C                    71.66       1     71.66     14.22      .002
 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
 Observed Power at the .0500 Level
                         Noncen-
 Source of Variation     trality   Power

 C                          .210    .064
 A BY C                   14.223    .938

* * * * * * A n a l y s i s   o f   V a r i a n c e -- design   1 * * *

 Tests of Significance for FMD using UNIQUE sums of squares
 Source of Variation          SS      DF        MS         F  Sig of F

 Error 4                  110.29      28      3.94
  C BY B                   11.35       2      5.68      1.44      .254
 A BY B BY C                3.18       2      1.59       .40      .672
```

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 1. (Continued) GROUP X INTENSITY X TIME (FMD)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 2 * * *

Tests of Significance for FMD using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	596.89	28	21.32		
A W C(1)	33.65	1	33.65	1.58	.219
A W C(2)	38.08	1	38.08	1.79	.192

* * * * * * A n a l y s i s o f V a r i a n c e -- design 3 * * *

Tests of Significance for FMD using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	70.54	14	5.04		
C W A(1)	45.07	1	45.07	8.95	.010
C W A(2)	27.65	1	27.65	5.49	.034

Observed Power at the .0500 Level

Source of Variation	Noncentrality	Power
C W A(1)	8.945	.793
C W A(2)	5.487	.586

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 2. GROUP X INTENSITY X TIME (BASELINE DIAMETER)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for BLDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	13.97	14	1.00		
A	.21	1	.21	.21	.654
Error 2	1.90	28	.07		
B	.06	2	.03	.43	.658
A BY B	.01	2	.01	.08	.921
Error 3	.58	14	.04		
C	.01	1	.01	.35	.566
A BY C	.07	1	.07	1.74	.209
Error 4	.54	28	.02		
C BY B	.31	2	.16	8.16	.002
A BY B BY C	.02	2	.01	.50	.614

* * * * * * A n a l y s i s o f V a r i a n c e -- design 2 * * *

Tests of Significance for BLDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	2.44	56	.04		
B W C(1)	.22	2	.11	2.49	.092
B W C(2)	.15	2	.08	1.77	.180

* * * * * * A n a l y s i s o f V a r i a n c e -- design 3 * * *

Tests of Significance for BLDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	.54	28	.02		
C W B(1)	.10	1	.10	5.22	.030
C W B(2)	.00	1	.00	.13	.725
C W B(3)	.23	1	.23	11.73	.002

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 3. GROUP X INTENSITY X TIME (HYPEREMIC DIAMETER)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	15.06	14	1.08		
A	.28	1	.28	.26	.617
Error 2	2.87	28	.10		
B	.07	2	.04	.36	.703
A BY B	.04	2	.02	.21	.812
Error 3	.61	14	.04		
C	.02	1	.02	.37	.553
A BY C	.00	1	.00	.00	.982
Error 4	.76	28	.03		
C BY B	.24	2	.12	4.41	.022
A BY B BY C	.04	2	.02	.71	.500

* * * * * * A n a l y s i s o f V a r i a n c e -- design 2 * * *

Tests of Significance for HDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	3.62	56	.06		
B W C(1)	.19	2	.10	1.48	.236
B W C(2)	.12	2	.06	.93	.401

* * * * * * A n a l y s i s o f V a r i a n c e -- design 3 * * *

Tests of Significance for HDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	.76	28	.03		
C W B(1)	.04	1	.04	1.63	.212
C W B(2)	.00	1	.00	.03	.867
C W B(3)	.21	1	.21	7.77	.009

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 4. GROUP X INTENSITY X TIME (BASELINE VELOCITY)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for BLFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	2734.69	14	195.33		
A	473.04	1	473.04	2.42	.142
Error 2	548.57	28	19.59		
B	4.56	2	2.28	.12	.890
A BY B	117.15	2	58.57	2.99	.067
Error 3	447.77	14	31.98		
C	222.96	1	222.96	6.97	.019
A BY C	9.44	1	9.44	.30	.596
Error 4	638.65	28	22.81		
C BY B	50.07	2	25.03	1.10	.348
A BY B BY C	10.92	2	5.46	.24	.789

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 5. GROUP X INTENSITY X TIME (HYPEREMIC VELOCITY)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	17498.18	14	1249.87		
A	6489.53	1	6489.53	5.19	.039
Error 2	7524.64	28	268.74		
B	158.71	2	79.36	.30	.747
A BY B	469.67	2	234.83	.87	.428
Error 3	1903.99	14	136.00		
C	563.09	1	563.09	4.14	.061
A BY C	979.84	1	979.84	7.20	.018
Error 4	4846.09	28	173.07		
C BY B	375.33	2	187.66	1.08	.352
A BY B BY C	702.22	2	351.11	2.03	.150

* * * * * * A n a l y s i s o f V a r i a n c e -- design 2 * * *

Tests of Significance for HFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	19402.18	28	692.93		
A W C(1)	1213.04	1	1213.04	1.75	.197
A W C(2)	6256.33	1	6256.33	9.03	.006

* * * * * * A n a l y s i s o f V a r i a n c e -- design 3 * * *

Tests of Significance for HFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	1903.99	14	136.00		
C W A(1)	28.68	1	28.68	.21	.653
C W A(2)	1514.25	1	1514.25	11.13	.005

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 6. GROUP X INTENSITY X TIME (HYPEREMIC LOCAL SHEAR STRESS)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HLSS using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	148.46	14	10.60		
A	23.03	1	23.03	2.17	.163
Error 2	41.51	28	1.48		
B	1.12	2	.56	.38	.690
A BY B	2.64	2	1.32	.89	.422
Error 3	11.57	14	.83		
C	3.31	1	3.31	4.00	.065
A BY C	3.48	1	3.48	4.21	.059
Error 4	26.76	28	.96		
C BY B	2.58	2	1.29	1.35	.275
A BY B BY C	2.58	2	1.29	1.35	.276

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 7. GROUP X INTENSITY X TIME (IL-6)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for IL6 using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	54.00	14	3.86		
A	5.33	1	5.33	1.38	.259
Error 2	13.67	28	.49		
B	7.24	2	3.62	7.42	.003
A BY B	.14	2	.07	.15	.863
Error 3	3.75	14	.27		
C	6.93	1	6.93	25.85	.000
A BY C	.12	1	.12	.45	.511
Error 4	2.67	28	.10		
C BY B	5.18	2	2.59	27.17	.000
A BY B BY C	.24	2	.12	1.25	.303

* * * * * * A n a l y s i s o f V a r i a n c e -- design 2 * * *

Tests of Significance for IL6 using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	16.33	56	.29		
B W C(1)	.20	2	.10	.33	.717
B W C(2)	12.22	2	6.11	20.95	.000

* * * * * * A n a l y s i s o f V a r i a n c e -- design 3 * * *

Tests of Significance for IL6 using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	2.67	28	.10		
C W B(1)	.02	1	.02	.19	.669
C W B(2)	2.60	1	2.60	27.32	.000
C W B(3)	9.49	1	9.49	99.61	.000

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 8. GROUP X INTENSITY X TIME (TNF-α)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for TNFa using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	5.92	14	.42		
A	.28	1	.28	.65	.433

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for TNFa using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 2	1.97	28	.07		
B	.30	2	.15	2.16	.134
A BY B	.08	2	.04	.58	.564

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for TNFa using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 3	.20	14	.01		
C	.00	1	.00	.31	.584
A BY C	.00	1	.00	.00	.983

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for TNFa using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 4	.20	28	.01		
C BY B	.02	2	.01	1.37	.270
A BY B BY C	.02	2	.01	1.71	.200

A= GROUP (active, inactive), B=INTENSITY (low, moderate, high), AND C = TIME (pre-exercise, 1hr post)

TABLE 9. INDEPENDENT SAMPLES T-TEST FOR ACTIVE VS INACTIVE (EXERCISE VARIABLES)

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
RPE	Equal variances assumed	4.558	.051	.814	14	.429	.33750	.41440	-.55131	1.22631
HR	Equal variances assumed	.852	.372	2.656	14	.019	12.25000	4.61268	2.35679	22.14321
SBP	Equal variances assumed	1.184	.295	-.416	14	.684	-3.25000	7.81653	-20.01480	13.51480
DBP	Equal variances assumed	.041	.842	.809	14	.432	4.50000	5.55934	-7.42360	16.42360
RPE50	Equal variances assumed	.003	.954	-.095	14	.926	-.07500	.79167	-1.77296	1.62296
HR50	Equal variances assumed	1.050	.323	1.366	14	.193	8.50000	6.22029	-4.84119	21.84119
SBP50	Equal variances assumed	.050	.826	1.270	14	.225	10.25000	8.06973	-7.05785	27.55785
DBP50	Equal variances assumed	3.332	.089	1.045	14	.314	4.00000	3.82660	-4.20724	12.20724
RPE75	Equal variances assumed	.088	.771	.707	14	.491	.45000	.63626	-.91463	1.81463
HR75	Equal variances assumed	.018	.894	.627	14	.541	3.75000	5.97726	-9.06996	16.56996
SBP75	Equal variances assumed	.073	.791	.662	14	.519	7.00000	10.57773	-15.68698	29.68698
DBP75	Equal variances assumed	.031	.864	1.053	14	.310	5.25000	4.98525	-5.44229	15.94229

TABLE 10. TRIAL X TIME (FMD)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for FMD using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	37.22	8	4.65		
S	261.25	8	32.66	7.02	.006
A	.03	1	.03	.01	.942
Error 2	225.74	32	7.05		
B	58.37	4	14.59	2.07	.108
Error 3	103.07	32	3.22		
B BY A	5.22	4	1.30	.41	.804

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 11. TRIAL X TIME (BASELINE DIAMETER)

* * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for BLDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	.44	8	.06		
S	13.94	8	1.74	31.60	.000
A	.02	1	.02	.39	.549
Error 2	.25	32	.01		
B	.07	4	.02	2.20	.091
Error 3	.14	32	.00		
B BY A	.00	4	.00	.14	.964

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 12. TRIAL X TIME (HYPEREMIC DIAMETER)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HDIAM using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	.42	8	.05		
S	15.95	8	1.99	38.20	.000
A	.05	1	.05	.94	.360
Error 2	.30	32	.01		
B	.14	4	.03	3.71	.014
Error 3	.23	32	.01		
B BY A	.02	4	.01	.77	.552

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 13. TRIAL X TIME (BASELINE VELOCITY)

* * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for BLFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	312.49	8	39.06		
S	1578.43	8	197.30	5.05	.017
A	16.38	1	16.38	.42	.535
Error 2	920.62	32	28.77		
B	2911.97	4	727.99	25.30	.000
Error 3	610.34	32	19.07		
B BY A	78.75	4	19.69	1.03	.406

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 14. TRIAL X TIME (HYPEREMIC VELOCITY)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HFLOW using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	737.27	8	92.16		
S	16824.31	8	2103.04	22.82	.000
A	58.73	1	58.73	.64	.448
Error 2	4347.42	32	135.86		
B	1848.07	4	462.02	3.40	.020
Error 3	4245.40	32	132.67		
B BY A	1069.80	4	267.45	2.02	.116

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 15. TRIAL X TIME (HYPEREMIC LOCAL SHEAR STRESS)

* * * * * * A n a l y s i s o f V a r i a n c e -- design 1 * * *

Tests of Significance for HLSS using UNIQUE sums of squares

Source of Variation	SS	DF	MS	F	Sig of F
Error 1	4.44	8	.56		
S	201.22	8	25.15	45.31	.000
A	.17	1	.17	.31	.594
Error 2	19.44	32	.61		
B	7.30	4	1.82	3.00	.033
Error 3	23.22	32	.73		
B BY A	5.41	4	1.35	1.86	.141

A= TRIAL (TRIAL 1, TRIAL 2), B=TIME (PRE-EXERCISE, IMMEDIATELY, 1HR POST, 2HR POST, 3 HR POST)

TABLE 16. INTRACLASS CORRELATIONS (PRE EXERCISE FMD)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.602	.604	2

Inter-Item Correlation Matrix

	Trial1a	Trial2a
Trial1a	1.000	.432
Trial2a	.432	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	7.370	6.973	7.767	.794	1.114	.315	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.431(b)	-.276	.835	2.513	8.0	8	.107
Average Measures	.602(c)	-.764	.910	2.513	8.0	8	.107

TABLE 17. INTRACLASS CORRELATIONS (IMMEDIATE FMD)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.840	.847	2

Inter-Item Correlation Matrix

	Trial1b	Trial2b
Trial1b	1.000	.734
Trial2b	.734	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	5.166	4.988	5.343	.355	1.071	.063	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.725(b)	.172	.931	6.270	8.0	8	.009
Average Measures	.840(c)	.293	.964	6.270	8.0	8	.009

TABLE 18. INTRACLASS CORRELATIONS (1HR POST FMD)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.632	.654	2

Inter-Item Correlation Matrix

	Trial1c	Trial2c
Trial1c	1.000	.486
Trial2c	.486	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	5.691	5.436	5.946	.510	1.094	.130	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.462(b)	-.239	.847	2.721	8.0	8	.089
Average Measures	.632(c)	-.629	.917	2.721	8.0	8	.089

TABLE 19. INTRACLASS CORRELATIONS (2HR POST FMD)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.724	.728	2

Inter-Item Correlation Matrix

	Trial1d	Trial2d
Trial1d	1.000	.573
Trial2d	.573	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	5.790	5.681	5.899	.218	1.038	.024	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.568(b)	-.100	.883	3.628	8.0	8	.043
Average Measures	.724(c)	-.222	.938	3.628	8.0	8	.043

TABLE 20. INTRACLASS CORRELATIONS (3HR POST FMD)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.631	.634	2

Inter-Item Correlation Matrix

	Trial1e	Trial2e
Trial1e	1.000	.464
Trial2e	.464	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	5.191	5.032	5.351	.319	1.063	.051	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.461(b)	-.241	.846	2.710	8.0	8	.090
Average Measures	.631(c)	-.636	.917	2.710	8.0	8	.090

TABLE 21. INTRACLASS CORRELATIONS (PRE-EXERCISE BASELINE DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.958	.960	2

Inter-Item Correlation Matrix

	Trial1a	Trial2a
Trial1a	1.000	.923
Trial2a	.923	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.106	4.085	4.128	.044	1.011	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.920(b)	.686	.981	23.845	8.0	8	.000
Average Measures	.958(c)	.814	.991	23.845	8.0	8	.000

TABLE 22. INTRACLASS CORRELATIONS (IMMEDIATE BASELINE DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.975	.976	2

Inter-Item Correlation Matrix

	Trial1b	Trial2b
Trial1b	1.000	.953
Trial2b	.953	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.133	4.118	4.147	.028	1.007	.000	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.952(b)	.804	.989	40.785	8.0	8	.000
Average Measures	.975(c)	.891	.994	40.785	8.0	8	.000

TABLE 23. INTRACLASS CORRELATIONS (1HR POST BASELINE DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.933	.935	2

Inter-Item Correlation Matrix

	Trial1c	Trial2c
Trial1c	1.000	.879
Trial2c	.879	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.146	4.128	4.164	.036	1.009	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.874(b)	.540	.970	14.838	8.0	8	.000
Average Measures	.933(c)	.701	.985	14.838	8.0	8	.000

TABLE 24. INTRACLASS CORRELATIONS (2HR POST BASELINE DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.949	.974	2

Inter-Item Correlation Matrix

	Trial1d	Trial2d
Trial1d	1.000	.949
Trial2d	.949	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.065	4.058	4.071	.012	1.003	.000	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.902(b)	.629	.977	19.491	8.0	8	.000
Average Measures	.949(c)	.773	.988	19.491	8.0	8	.000

TABLE 25. INTRACLASS CORRELATIONS (3HR POST BASELINE DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.979	.986	2

Inter-Item Correlation Matrix

	Trial1e	Trial2e
Trial1e	1.000	.972
Trial2e	.972	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.113	4.096	4.131	.035	1.009	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.959(b)	.831	.991	48.014	8.0	8	.000
Average Measures	.979(c)	.908	.995	48.014	8.0	8	.000

TABLE 26. INTRACLASS CORRELATIONS (PRE-EXERCISE HYPEREMIC DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.930	.934	2

Inter-Item Correlation Matrix

	Trial1a	Trial2a
Trial1a	1.000	.876
Trial2a	.876	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.411	4.360	4.462	.102	1.023	.005	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.869(b)	.527	.969	14.324	8.0	8	.001
Average Measures	.930(c)	.691	.984	14.324	8.0	8	.001

TABLE 27. INTRACLASS CORRELATIONS (IMMEDIATE HYPEREMIC DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.983	.983	2

Inter-Item Correlation Matrix

	Trial1b	Trial2b
Trial1b	1.000	.967
Trial2b	.967	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.343	4.326	4.360	.034	1.008	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.966(b)	.856	.992	57.308	8.0	8	.000
Average Measures	.983(c)	.923	.996	57.308	8.0	8	.000

TABLE 28. INTRACLASS CORRELATIONS (1HR POST HYPEREMIC DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.941	.942	2

Inter-Item Correlation Matrix

	Trial1c	Trial2c
Trial1c	1.000	.890
Trial2c	.890	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.380	4.355	4.405	.050	1.011	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.889(b)	.588	.974	17.093	8.0	8	.000
Average Measures	.941(c)	.741	.987	17.093	8.0	8	.000

TABLE 29. INTRACLASS CORRELATIONS (2HR POST HYPEREMIC DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.977	.980	2

Inter-Item Correlation Matrix

	Trial1d	Trial2d
Trial1d	1.000	.961
Trial2d	.961	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.302	4.281	4.323	.042	1.010	.001	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.954(b)	.812	.990	42.803	8.0	8	.000
Average Measures	.977(c)	.896	.995	42.803	8.0	8	.000

TABLE 30. INTRACLASS CORRELATIONS (3HR POST HYPEREMIC DIAMETER)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.966	.968	2

Inter-Item Correlation Matrix

	Trial1e	Trial2e
Trial1e	1.000	.938
Trial2e	.938	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	4.322	4.319	4.325	.006	1.001	.000	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.934(b)	.737	.985	29.240	8.0	8	.000
Average Measures	.966(c)	.848	.992	29.240	8.0	8	.000

TABLE 31. INTRACLASS CORRELATIONS (PRE-EXERCISE BASELINE VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.711	.716	2

Inter-Item Correlation Matrix

	TRIAL 1	TRIAL 2
TRIAL 1	1.000	.558
TRIAL 2	.558	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	21.372	20.922	21.822	.900	1.043	.405	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.552(b)	-.123	.878	3.462	8.0	8	.049
Average Measures	.711(c)	-.281	.935	3.462	8.0	8	.049

TABLE 32. INTRACLASS CORRELATIONS (IMMEDIATE BASELINE VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.577	.587	2

Inter-Item Correlation Matrix

	TRIAL 1	TRIAL 2
TRIAL 1	1.000	.415
TRIAL 2	.415	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	27.217	25.200	29.233	4.033	1.160	8.134	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.405(b)	-.305	.826	2.361	8.0	8	.123
Average Measures	.577(c)	-.877	.904	2.361	8.0	8	.123

TABLE 33. INTRACLASS CORRELATIONS (1HR POST BASELINE VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.560	.561	2

Inter-Item Correlation Matrix

	TRIAL 1	TRIAL 2
TRIAL 1	1.000	.390
TRIAL 2	.390	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	16.711	15.822	17.600	1.778	1.112	1.580	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.389(b)	-.322	.820	2.275	8.0	8	.133
Average Measures	.560(c)	-.949	.901	2.275	8.0	8	.133

TABLE 34. INTRACLASS CORRELATIONS (2HR POST BASELINE VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.543	.585	2

Inter-Item Correlation Matrix

	TRIAL 1	TRIAL 2
TRIAL 1	1.000	.414
TRIAL 2	.414	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	13.389	13.300	13.478	.178	1.013	.016	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.373(b)	-.339	.813	2.190	8.0	8	.144
Average Measures	.543(c)	-1.025	.897	2.190	8.0	8	.144

TABLE 35. INTRACLASS CORRELATIONS (3HR POST BASELINE VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.577	.644	2

Inter-Item Correlation Matrix

	TRIAL 1	TRIAL 2
TRIAL 1	1.000	.475
TRIAL 2	.475	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	11.478	11.011	11.944	.933	1.085	.436	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.405(b)	-.305	.826	2.362	8.0	8	.123
Average Measures	.577(c)	-.877	.905	2.362	8.0	8	.123

TABLE 36. INTRACLASS CORRELATIONS (PRE-EXERCISE HYPEREMIC VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.613	.613	2

Inter-Item Correlation Matrix

	Trial1a	Trial2a
Trial1a	1.000	.442
Trial2a	.442	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	95.006	93.800	96.211	2.411	1.026	2.907	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.442(b)	-.264	.839	2.581	8.0	8	.101
Average Measures	.613(c)	-.717	.913	2.581	8.0	8	.101

TABLE 37. INTRACLASS CORRELATIONS (IMMEDIATE HYPEREMIC VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.827	.827	2

Inter-Item Correlation Matrix

	Trial1b	Trial2b
Trial1b	1.000	.705
Trial2b	.705	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	89.994	89.522	90.467	.944	1.011	.446	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.705(b)	.131	.925	5.769	8.0	8	.011
Average Measures	.827(c)	.231	.961	5.769	8.0	8	.011

TABLE 38. INTRACLASS CORRELATIONS (1HR POST HYPEREMIC VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.767	.816	2

Inter-Item Correlation Matrix

	Trial1c	Trial2c
Trial1c	1.000	.689
Trial2c	.689	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	94.028	87.400	100.656	13.256	1.152	87.855	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.623(b)	-.015	.900	4.298	8.0	8	.027
Average Measures	.767(c)	-.031	.948	4.298	8.0	8	.027

TABLE 39. INTRACLASS CORRELATIONS (2HR POST HYPEREMIC VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.812	.846	2

Inter-Item Correlation Matrix

	Trial1d	Trial2d
Trial1d	1.000	.734
Trial2d	.734	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	85.122	84.378	85.867	1.489	1.018	1.108	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.684(b)	.091	.919	5.326	8.0	8	.015
Average Measures	.812(c)	.168	.958	5.326	8.0	8	.015

TABLE 40. INTRACLASS CORRELATIONS (3HR POST HYPEREMIC VELOCITY)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.770	.810	2

Inter-Item Correlation Matrix

	Trial1e	Trial2e
Trial1e	1.000	.680
Trial2e	.680	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	83.800	79.733	87.867	8.133	1.102	33.076	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.626(b)	-.010	.901	4.341	8.0	8	.027
Average Measures	.770(c)	-.021	.948	4.341	8.0	8	.027

TABLE 41. INTRACLASS CORRELATIONS (PRE-EXERCISE HYPEREMIC LOCAL SHEAR STRESS)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.854	.856	2

Inter-Item Correlation Matrix

	Trial1a	Trial2a
Trial1a	1.000	.748
Trial2a	.748	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	6.588	6.522	6.653	.130	1.020	.008	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.745(b)	.215	.936	6.856	8.0	8	.007
Average Measures	.854(c)	.353	.967	6.856	8.0	8	.007

TABLE 42. INTRACLASS CORRELATIONS (IMMEDIATE HYPEREMIC LOCAL SHEAR STRESS)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.931	.931	2

Inter-Item Correlation Matrix

	Trial1b	Trial2b
Trial1b	1.000	.871
Trial2b	.871	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	6.211	6.156	6.266	.110	1.018	.006	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.871(b)	.531	.969	14.472	8.0	8	.001
Average Measures	.931(c)	.694	.984	14.472	8.0	8	.001

TABLE 43. INTRACLASS CORRELATIONS (1HR POST HYPEREMIC LOCAL SHEAR STRESS)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.860	.889	2

Inter-Item Correlation Matrix

	Trial1c	Trial2c
Trial1c	1.000	.800
Trial2c	.800	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	6.450	6.020	6.881	.861	1.143	.371	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.755(b)	.235	.939	7.161	8.0	8	.006
Average Measures	.860(c)	.381	.969	7.161	8.0	8	.006

TABLE 44. INTRACLASS CORRELATIONS (2HR POST HYPEREMIC LOCAL SHEAR STRESS)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.865	.931	2

Inter-Item Correlation Matrix

	Trial1d	Trial2d
Trial1d	1.000	.871
Trial2d	.871	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	6.006	5.903	6.110	.207	1.035	.021	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.762(b)	.251	.941	7.401	8.0	8	.005
Average Measures	.865(c)	.401	.970	7.401	8.0	8	.005

TABLE 45. INTRACLASS CORRELATIONS (3HR POST HYPEREMIC LOCAL SHEAR STRESS)

Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.876	.936	2

Inter-Item Correlation Matrix

	Trial1e	Trial2e
Trial1e	1.000	.879
Trial2e	.879	1.000

Summary Item Statistics

	Mean	Minimum	Maximum	Range	Maximum / Minimum	Variance	N of Items
Item Means	5.805	5.479	6.131	.652	1.119	.213	2

Intraclass Correlation Coefficient

	Intraclass Correlation(a)	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.779(b)	.289	.945	8.043	8.0	8	.004
Average Measures	.876(c)	.449	.972	8.043	8.0	8	.004

TABLE 46. REPRODUCIBILITY CORRELATIONS (ALL FMD COMBINED)

		Trial1combo	Trial2combo
Trial1combo	Pearson Correlation	1	.579(**)
	Sig. (2-tailed)		.000
	N	45	45
Trial2combo	Pearson Correlation	.579(**)	1
	Sig. (2-tailed)	.000	
	N	45	45

** Correlation is significant at the 0.01 level (2-tailed).

TABLE 47. REPRODUCIBILITY CORRELATIONS (PRE-EXERCISE FMD)

		Trial1a	Trial2a
Trial1a	Pearson Correlation	1	.432
	Sig. (2-tailed)		.245
	N	9	9
Trial2a	Pearson Correlation	.432	1
	Sig. (2-tailed)	.245	
	N	9	9

TABLE 48. REPRODUCIBILITY CORRELATIONS (IMMEDIATE FMD)

		Trial1b	Trial2b
Trial1b	Pearson Correlation	1	.734(*)
	Sig. (2-tailed)		.024
	N	9	9
Trial2b	Pearson Correlation	.734(*)	1
	Sig. (2-tailed)	.024	
	N	9	9

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 49. REPRODUCIBILITY CORRELATIONS (1HR POST FMD)

		Trial1c	Trial2c
Trial1c	Pearson Correlation	1	.486
	Sig. (2-tailed)		.184
	N	9	9
Trial2c	Pearson Correlation	.486	1
	Sig. (2-tailed)	.184	
	N	9	9

TABLE 50. REPRODUCIBILITY CORRELATIONS (2HR POST FMD)

		Trial1d	Trial2d
Trial1d	Pearson Correlation	1	.573
	Sig. (2-tailed)		.107
	N	9	9
Trial2d	Pearson Correlation	.573	1
	Sig. (2-tailed)	.107	
	N	9	9

TABLE 51. REPRODUCIBILITY CORRELATIONS (3HR POST FMD)

		Trial1e	Trial2e
Trial1e	Pearson Correlation	1	.464
	Sig. (2-tailed)		.209
	N	9	9
Trial2e	Pearson Correlation	.464	1
	Sig. (2-tailed)	.209	
	N	9	9

APPENDIX E – HUMAN SUBJECTS REVIEW AND APPROVAL

HUMAN SUBJECTS NOTICE OF APPROVAL

INDIANA UNIVERSITY

NOTICE OF APPROVAL
FULL COMMITTEE REVIEW

TO: Ryan Harris
HPER

DATE: November 15, 2005

FROM: Human Subjects Risk Compliance

RE: Protocol entitled: The Effect of Acute Exercise on Endothelial Function: A Mechanistic Approach
Protocol #: 05-10379

The Human Subjects Committee (HSC) has reviewed and approved the research protocol referenced above. As the principal investigator of this study you assume the following reporting responsibilities:

CONTINUING REVIEW: A status report must be filed with the committee. You are required to apply for renewal of approval at least once a year for as long as the study is active. All projects will automatically receive a renewal notice from the HSC. **This study is approved from November 15, 2005 to November 14, 2006.**

AMENDMENTS: Investigators are required to report on these forms **ANY** changes to the research study (such as design, procedures, consent forms, or subject population, including size). An amendment form is attached for your future use. ***The new procedure may not be initiated until HSC approval has been given.***

AUDIT OR INSPECTION REPORTS: Investigators are required to provide to the HSC a copy of any audit or inspection reports or findings issued to them by regulatory agencies, cooperative research groups, contract research organizations, the sponsor, or the funding agency.

COMPLETION: You are required to notify the HSC office when your study is completed (data analysis finished). Please contact the HSC office for the appropriate form to use.

CONSENT FORMS: All subjects should be given a copy of the **stamped approved** consent form. You must retain signed consent documents for at least three years past completion of the research activity.

We suggest you keep this letter with your copy of the approved protocol. Please refer to the exact project title and protocol number in any future correspondence with our office. All correspondence must be typed.

Enclosures: Documentation of Review and Approval
Amendment Form
Approved Consent Form- **stamped copy must be used**

Federal Wide Assurance #FWA00003544-IRB00000222
For additional FWA information, see the Web site at http://www.iupui.edu/~resgrad/spon/fwa.htm

OFFICE OF THE VICE PRESIDENT FOR RESEARCH

BLOOMINGTON CAMPUS COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS

Location:
Indiana University
Carmichael Center L03
530 East Kirkwood Avenue
Bloomington, Indiana

Mailing Address:
P.O. Box 1847
Bloomington, Indiana
47402

812-855-3067
Fax: 812-856-1535
E-mail:
iub_hsc@indiana.edu
WWW Address:
http://www.research.indiana.edu/rschcomp/hsrpg.html

HUMAN SUBJECTS NOTICE OF CONTINUING REVIEW

INDIANA UNIVERSITY

NOTICE OF APPROVAL
CONTINUING REVIEW
FULL COMMITTEE REVIEW

OFFICE OF THE VICE PRESIDENT FOR RESEARCH

TO: Ryan Harris
HPER

DATE: October 23, 2006

FROM: Human Subjects Risk Compliance

RE: Protocol entitled: The Interaction of TNF-Alpha, Interleukin-6, and Acute Exercise on Endothelial Function
Protocol #: 05-10379

The Human Subjects Committee (HSC) has reviewed and approved the Continuing Review for the research protocol referenced above. As the principal investigator of this study you assume the following reporting responsibilities:

CONTINUING REVIEW: A status report must be filed with the committee. You are required to apply for renewal of approval at least once a year for as long as the study is active. The HSC will attempt to notify you by e-mail before approval expires, but it is ultimately your responsibility to make sure your approval does not expire. **This study is approved from October 19, 2006 to October 18, 2007.**

AMENDMENTS: Investigators are required to report on the Study Amendment Form **ANY** changes to the research study (such as design, procedures, study information sheet/consent form, or subject population, including size). **Changes may not be initiated until HSC approval has been given.** The PDF interactive Study Amendment Form may be found at: http://www.research.indiana.edu/rschcomp/pdf/amendpdf2.pdf

AUDIT OR INSPECTION REPORTS: Investigators are required to provide to the HSC a copy of any audit or inspection reports or findings issued to them by regulatory agencies, cooperative research groups, contract research organizations, the sponsor, or the funding agency.

COMPLETION: You are required to notify the HSC office when your study is completed (data analysis finished). Please contact the HSC office for the appropriate form to use.

ADVERSE REACTIONS: If any unexpected adverse reactions occur as a result of this study, you must notify the HSC office immediately. A written report must be filed within 3 working day.

CONSENT FORMS: All subjects should be given a copy of **the stamped approved** consent form. You must retain signed consent documents for at least three years past completion of the research activity.

We suggest you keep this letter with your copy of the approved protocol. Please refer to the exact project title and protocol number in any future correspondence with our office. All correspondence must be typed.

Enclosures: Continuing Review Approval
Approved Consent Form- **stamped copy must be used**

Federal Wide Assurance #FWA00003544-IRB00000222
For additional FWA information, see the Web site at http://www.iupui.edu/~resgrad/spon/fwa.htm

BLOOMINGTON CAMPUS COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS

Location:
Indiana University
Carmichael Center L03
530 East Kirkwood Avenue
Bloomington, Indiana

Mailing Address:
P.O. Box 1847
Bloomington, Indiana
47402

812-855-3067
Fax: 812-856-1535
E-mail:
iub_hsc@indiana.edu
WWW Address:
http://www.research.indiana.edu/rschcomp/limpg.html

HUMAN SUBJECTS PROPOSAL

INDIANA UNIVERSITY
BLOOMINGTON CAMPUS COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS

DOCUMENTATION OF REVIEW AND APPROVAL
of
Research Project Utilizing Human Subjects

Study #

TITLE OF PROJECT The Effect of Acute Exercise on Endothelial Function: A Mechanistic Approach

PROJECT DURATION - START DATE November 2005 END DATE December 2006

PRIN. INVESTIGATOR Ryan Harris SCHOOL/DEPARTMENT HPER/Clinical Exercise Physiology

ADDRESS 1025 E. 7th Street HPER 070 E-MAIL Harrisra@indiana.edu Phone: 812-855-7556

RANK: Faculty ____ Res. Scientist ____ Post-Doc ____ Staff ____ Student: undergrad masters ____ PhD/EdD_X

If PI's rank is OTHER than faculty, name of faculty overseeing the research (Sponsor) Janet P. Wallace PhD.

SPONSOR'S E-MAIL & CAMPUS ADDRESS wallacej@indiana.edu, HPER 112, PHONE 855-6384

FUNDING AGENCY Gatorade Sports Science Institute APPL. DEADLINE June 2005

AGENCY PROJECT # Student Grant Proposal 2005 **New**

As the principal investigator, my signature testifies that I pledge to conform to the following:

As one engaged in investigation utilizing human subjects, I acknowledge the rights and welfare of the human subject involved.

I acknowledge my responsibility as an investigator to secure the informed consent of the subject by explaining the procedures, in so far as possible, and by describing the risks as weighed against the potential benefits of the investigation.

I assure the Committee that all procedures performed under the project will be conducted in accordance with those Federal regulations and University policies which govern research involving human subjects. **Any deviation from the project (e.g., change in principal investigator, research methodology, subject recruitment procedures, etc.) will be submitted to the Committee in the form of an amendment for its approval prior to implementation.**

PRINCIPAL INVESTIGATOR:
Ryan Harris _____
(typed/printed name) (signature

As the faculty sponsor, my signature testifies that I have reviewed this application and that I will oversee the research in its entirety, through the termination report.

FACULTY SPONSOR:

Janet P.Wallace _____
(typed/printed name) (signature)

CAMPUS LEVEL REVIEW

This protocol for the use of human subjects has been reviewed and approved by the Indiana University/Bloomington Campus Committee for the Protection of Human Subjects.

SUMMARY SAFEGUARD STATEMENT

Project Title (if you wish to use a different title in the consent statement than is listed on page 3, explain here):

The Effect of Acute Exercise on Endothelial Function: A Mechanistic Approach

IF ADDITIONAL SPACE FOR RESPONSES IS DESIRED, THIS DOCUMENT MAY BE RETYPED ONTO PLAIN PAPER MAINTAINING THE IDENTICAL ORDER AND EXACT QUESTION WORDING WHILE ADDING EXTRA SPACE WHERE NEEDED.

Do not type on the reverse side of any form.
Use type size no smaller than ARIAL 11 or TIMES NEW ROMAN 12 point.

A. Briefly describe, in lay terms, the general nature and purpose of the proposed research, and where the study will take place. If student research, indicate whether for a course, thesis, dissertation, or independent research. If the study is only for a course, please review the Student Research Policy to ascertain if this project requires HSC review.

Endothelial cells, located between the circulating blood and the vessel wall, participate in multiple essential functions including, regulation of smooth muscle tone, thrombosis control, and platelet aggregation control. A dysfunctional endothelium has been related to an increase risk for cardiovascular disease in clinical populations. Flow-Mediated Dilation (FMD) is a non-invasive measurement of endothelial function which reflects the overall health and function of the arteries. Research suggests a single bout of exercise; 60% VO2max for 45 minutes, increases arterial function approximately 1 hr post intervention; however, the mechanisms related to these findings are not fully understood.

Inflammation has been found to induce endothelial dysfunction. TNF-alpha is a pro-inflammatory biomarker that has been associated with the pathogenesis of cardiovascular disease as well as inducing endothelial dysfunction by decreasing the blood flow. Exercise has been found to inhibit this inflammatory process; however, the role of TNF-alpha as a mechanism is unknown.

There are 2 phases associated with the proposed investigation. Each subject will only participate in one of the following phases. The purpose of phase 1 is to describe the effect of endothelial function for up to six hours following an acute bout of exercise. The purpose of phase 2 is to investigate the dose-response relationship of exercise on endothelial function and investigate TNF-alpha as a proposed mechanism. It is hypothesized that 1) The greater volume of exercise is associated with a larger increase in brachial artery reactivity, and 2) TNF-alpha as an inflammatory mediator plays a major role in brachial artery reactivity.

The proposed study is intended to be my dissertation and will take place in the Clinical Exercise Physiology Laboratory in the school of Health Physical Education and Recreation.

B. Describe the process by which subjects will be recruited (see item F on page 2), how many (or estimate) subjects will be involved in the research, and how much time will be required of them. List specific eligibility requirements for subjects (or describe screening procedures), including those criteria that would exclude otherwise acceptable subjects. If your study uses only male or female subjects, explain why. For NIH-funded research only, address the inclusion of women, minorities and children in the research. Disclose any relationship between researcher and subjects - such as, teacher/student; superintendent/principal/teacher; employer/employee (see Students as Subjects section in the Policy Manual).

Approximately 20-30 moderate risk adults (18-60yrs) will be asked to participate in this study with approval from their physician and no financial compensation. Moderate risk, suggested by the American College of Sports Medicine, are those men who are $\geq$ 45 years old or women $\geq$ 55 years old or any individuals who meet the threshold for two or more risk factors (ie: hypertension (BP$\geq$140/90), hypercholesterolemia (TC>200mg/dl), smoking, sedentary, family history of heart disease, obesity (BMI$\geq$30kg/m^2), and impaired fasting glucose(BG 100-125mg/dl)) for coronary heart disease. Majority of the subjects will be recruited from the Adult Fitness Program at Indiana University. Additional subjects will be adult volunteers recruited from the University and within the Bloomington community. Participation for this study will take approximately 16-20 hours over a period of 4 non consecutive days. Subjects included in the study will be moderate risk identified by risk stratification, and will have their physicians approval. Subjects will be excluded if they

- are not between 18 and 60 years of age,
- do not fall into the moderate risk classification (as described above in section B),
- cannot exercise at 75% VO_2peak for 45 minutes,
- have a known allergy to heparin,
- do not have peripheral venous access in the left upper extremity
- have cardiovascular, pulmonary or metabolic diseases
- have gallbladder disease
- have orthopedic problems that limit your exercise
- take medications that dilate your arteries such as nitroglycerin, prostacyclin, or verapamil

Subjects will be instructed to 1) abstain from exercise or any unnecessary physical activity for 12 hours, 2) fast for 8 hours, 3) abstain from vitamin supplementation, tobacco, and caffeine for 8 hours, all prior to each day of the investigation.

C. Check appropriate box for type of vulnerable subject population involved when investigation specifically studies:
[_] minors (under age 18), [_] fetuses, [_] pregnant women, [_] persons with mental disabilities,
[_] prisoners, [_] persons with physical disabilities, [_] economically or educationally disadvantaged,
[_] other vulnerable population.

If any of the above are used, state the necessity for doing so. Please indicate the approximate age range of the minors to be involved.

No Vulnerable subject populations will be used.

D. List all procedures to be used on human subjects or describe what subjects will do. If done during regular class time, explain what non-participants will do. If you are taping, explain that here (see item 13 on page 11). *Asterisk* those you consider experimental. For those asterisked procedures, describe the usual method(s), if any, that were considered and why they were not used. (See item F on page 2 for more information.)

1. The **risk stratification form** summarizes information about the subject's medical history, present diseases/disorders, family history of disease, height and weight, and current medications along with information that will be obtained from each subjects fasting blood draw. If there is any question regarding any information presented in the risk stratification form, the subjects physician may be consulted.
2. A **fasting blood draw** performed at the Indiana University Health center will be required before any subject can participate in the study. This test is to help determine the risk stratification for exercise. Each subject will report to the Indiana University Health Center (Room 208) and must be fasting for at least 12 hours prior to this test. A 20-45 ml sample of venous blood will be drawn by a certified technician via sterile techniques for analysis of cholesterol, triglycerides and glucose. This test will take approximately 10-15 minutes.
3. A **maximal graded exercise test** will be performed to obtain the appropriate exercise treatment levels for each phase of the proposed investigation. This test is designed to measure the subjects exercise capacity. Each subject will walk or jog on a motor-driven treadmill beginning at a speed between 2.5-5.0 mph, 0% grade, with the grade or slope increasing 2.5% every two minutes until volitional fatigue, breathlessness, chest discomfort, and/or any other symptoms which indicate to yourself or the technicians that you should stop exercise. Heart rate via 12-lead EKG will be monitored throughout the test. Blood pressure will be taken before, during if walking (every stage), and after the test (recovery). Expired gases will also be collected through a unidirectional flow mouthpiece during the test. The total duration of this test is approximately 45 minutes.
4. If the subject is participating in phase 1, they will be asked to perform a single 45 minute **walking exercise session** at 60% of their exercise capacity (obtained from the maximal graded exercise test). Following this session of exercise, FMD will be performed immediately and every hour thereafter for six hours. They will be asked to remain in the laboratory for the six hour recovery period. During this time they will be asked to perform sedentary work which may include, using computers, doing work, reading, etc.
   Phase 2: If the subject is participating in phase 2, they will be asked to perform three separate **walking exercise session**s at 25%, 60%, and 75% of their exercise capacity (on three separate days) obtained from the maximal graded exercise test. Following this acute bout of exercise, FMD will be performed approximately three times based on the findings of phase 1 of this study. Phase 1 and 2 - Expired gases will be measured through a unidirectional flow mouthpiece during

the 5th minute of each exercise session to confirm their exercise intensity. The work rate will be adjusted if it is not within ±10% of your target exercise intensity. Expired gases will then be measured again during the 15th minute to confirm the new exercise intensity. Heart rate and blood pressure will be monitored throughout each exercise session.

5. **Flow-mediated dilation** (a non-invasive procedure) is used to characterize the health of the arteries. Each subject will lie on their back for 20 minutes to establish a resting state. After the 20 minute acclimation phase, a water based silicon gel will be placed on the ultrasound transducer as an impedance adapter for better ultrasound images. The Sonoace Pico ultra sound system using a 7.0 MHz linear transducer will then be used to scan the baseline artery of their inner arm, 2-10 cm above the elbow. Once a clear image is obtained, resting blood flow velocity and a baseline image of their artery will be captured by the ultrasound. After baseline measurements are captured, a second blood pressure cuff will be wrapped around the forearm of the right arm and inflated to 250 mm Hg to stop blood flow to the lower arm for 5 minutes. Blood flow velocity will be recorded 15 seconds after the cuff is deflated and ultrasound images will be captured as described above. The health of the artery will be expressed as a percentage of the artery expansion with the increased blood flow, compared to the resting diameter. **The dependent variables will be brachial artery diameter(mm) and absolute change in brachial artery diameter(mm)**.
6. Phase 2 only: For the **continuous blood draw**, a 24 gauge venous catheter will be inserted in a vein in the subjects left arm by a clinical exercise physiologist who has special IV training and practice. The catheter will remain there for the entire duration of the testing day (approximately 6-8 hrs). For each measurement of FMD performed during phase 2 of the study, the catheter will be flushed with normal saline, and a small blood sample (15-20ml) will be collected from the inserted catheter and stored for the analysis of TNF-alpha; a biomarker linked to inflammation and arterial health. Any period of time lasting longer than 2 hours between sample collections will require the IV to be flushed with a heparin based solution to prevent clotting of the catheter. Once the testing day is completed, the catheter will be removed by the same trained clinical exercise physiologist. **The dependent variable will be plasma concentrations of TNF-alpha (pg/ml).**

E. State the potential risks - for example, physical, psychological, financial, social, legal or other - connected with the proposed procedures.

Briefly describe how risks to subjects are reasonable in relation to anticipated benefits. Describe procedures for protecting against, or minimizing, potential risks. Assess their likely effectiveness. If you are using an electrical device that is attached directly to subjects explain how the subjects will be protected from shock.

The risks associated with the entire study are minimal.

1. There are no risks associated with filling out the **risk stratification form.**
2. The risks associated with a **fasting blood draw** may include fainting, soreness, bruising, and/or swelling at the venipuncture site. This risk is minimized by

having the blood drawn by a trained technician while you are in a seated position at the IU Health Center.

3. The risks involved with **maximal graded exercise testing** can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats and abnormal blood pressure responses. The risk of a serious event, although rare (sudden death = 1/10,000; heart attack = 4/10,000 tests; and complications requiring hospitalization = 10/10,000) does exist. Reasonable effort will be made to conduct the test in such a way as to minimize your discomfort and risk. The mouthpieces will be sanitized with the proper disinfecting detergents. In the unlikely event of an emergency we will call Bloomington Hospital Ambulance Service. The laboratory is equipped to respond to such situations and its personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.
4. The risks involved with sub-maximal **exercise sessions** can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats, and abnormal blood pressure responses. The risk of an event, although rare, is significantly reduced from exercise testing because of the sub-maximal intensity (morbidity = 1/887,526 participant hours of exercise and mortality = 1/1,124,000 participant hours). Reasonable effort will be made to minimize risk through prior screening and the use of proper warm-up, exercise and cool-down technique. The mouthpiece will be sanitized with the proper disinfecting detergents. In the unlikely event of a heart related emergency we will call the Bloomington Hospital Ambulance Service. The laboratory is equipped to respond to such situations and its personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.
5. The risks associated with forearm occlusion when measuring **FMD** may include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with **ultrasound measurements** may include skin irritation and pressure around the transducer sites. The risks associated with the **Ultrasound lubricant gel** are skin irritation and possible break out of rash.
6. The risks associated with the **blood draw via angiocath** may include infection, allergic reaction to heparin, irritation and bruising of the skin, pain, discomfort, collapsed vein, multiple puncture sites, and fainting. This risk is minimized by having the catheter inserted in a supine or seated position. If a mild reaction (ie. local swelling or redness at site) to heparin occurs, oral Benadryl® will be provided. If a serious reaction (ie. rash, swelling of the face, anaphylaxis) the emergency protocol will be initiated. This risk, although rare, is minimized by using proper sterile technique. There is a chance of phlebitis which can cause redness, swelling, moderate discomfort, and fever for up to a few days after the catheter is removed. If mild symptoms of phlebitis develop, warm compresses, alternating Tylenol® and Advil® will be indicated till the symptoms are gone (usually 12-72 hours). If serious

symptoms of phlebitis develop (ie. fever or red streaks up the arm) the subject will be instructed to go to prompt care or the ER immediately. This risk is minimized by using proper sterile technique.

Every effort will be made to minimize these discomforts.

F. Describe methods for preserving confidentiality. How will data be recorded and stored, with or without identifiers? If identifiers are used describe the type: names, job titles, number code, etc. How long are identifiers kept? If coding system is used, is there a link back to the subject's ID? If yes, where is the code list stored in relation to data and when is the code list destroyed? How will reports will be written, in aggregate terms, or will individual responses be described? Will subjects be identified in reports (see item 5 on page 10)? Describe disposition of tapes/films at the end of the study. If tapes are to be kept, indicate for how long and describe future uses of tapes.

All laboratory data will be collected in the privacy of the Clinical Exercise Physiology Laboratory. All data will be coded and filed with the reference to the subjects' participant number. Codes are stored in the graduate office of the clinical exercise physiology lab in a card file separate from the data files. All data will be stored in files locked in the Clinical Exercise Physiology Laboratory. Any part of the results obtained throughout this investigation will be made available to the subjects referring physician if desired by the subject. No subject will be referred to by name in any publication or summary of this work. Data collected will be stored and kept by your research participation number and may be used for future research studies. These data will be kept indefinitely because they contain medical related information. The subject is free to withdraw from the study at any time.

G. What, if any, benefit is to be gained by the subject? In the event of monetary gain, include all payment arrangements (amount of payment and the proposed method of disbursement), including reimbursement of expenses. If class credit will be given, list the amount and the value as it relates to the total points needed for an A. List alternative ways to earn the same amount of credit. If merchandise or a service is given, indicate the value. Explain the amount of partial payment/class credit if the subject withdraws prior to completion of the study. (See policy at http://www.indiana.edu/~resrisk/compensation.html)

The benefits of this study outweigh the risks. This new non-invasive technique to measure the health of the artery may provide further insight on the development and treatment of disease. Using exercise as a non-pharmacological intervention may create a safer and more effective treatment in prevention and management of disease. This study is one of many that will aid in discovering the most efficient exercise treatment in disease.

H. What information may accrue to science or society in general as a result of this work?

Endothelial function and arterial stiffness are two independent risk factors of cardiovascular disease. Cardiovascular disease is the leading killer in the United States. If we can identify risks of cardiovascular disease at an early stage, we can provide the

most effective dose of exercise as a treatment and possible delay or prevent the disease from progressing.

I. Coinvestigators, Cooperating Departments, Cooperating Institutions. If there are multiple investigators, please indicate only one person on the Documentation of Review and Approval (page 3) as the principal investigator; others should be designated as coinvestigators here. **Coinvestigators, not signing on page 3, should sign here, pledging to conform to the sentences on page 3.** If you anticipate that another department or institution may be involved in this research, list that here. If you are working with another institution, please include a letter of cooperation from that institution.
Please provide the person's name and e-mail address.

A. Co investigators will include:

a. Janet P. Wallace, PhD.
Email: wallacej@indiana.edu

b. Larry D. Rink, MD
Email: lrink@ima-md.com

INFORMED CONSENT (Inactive)

INDIANA UNIVERSITY - BLOOMINGTON
Informed Consent Statement

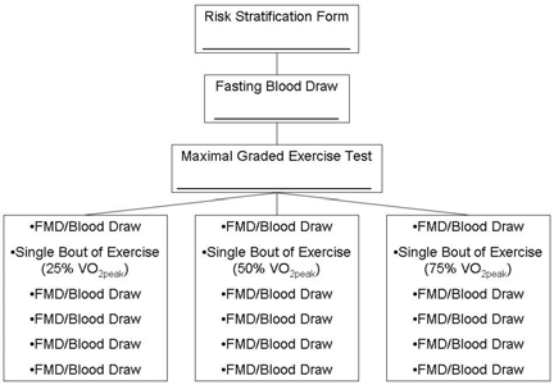
The Interaction of TNF-α, IL-6, and Acute Exercise on Endothelial Function

You are invited to participate in a research project under the supervision of Janet P. Wallace, Ph.D., Ryan Harris, M.S., and Larry Rink, M.D., at Indiana University. The purpose of this study is a) to investigate how the exercise intensity is related to the health of your arteries, and b) to investigate whether the exercise effects are related to the biomarker TNF-alpha, a marker of inflammation.

RESEARCH DESIGN INFORMATION

There will be approximately 15-20 subjects recruited for this investigation. Participation of this study will required you to 1) fill out a risk stratification form, 2) have fasting blood taken at the IU health center, and 3) perform a maximal graded exercise test on a separate day to determine the appropriate exercise intensity (25%, 50%, or 75% VO_2peak).

Flow-Mediated Dilation and TNF-alpha will be taken at baseline and post exercise sessions. Each subject will take approximately 2-3 weeks for completion of this study.



Note: Some answers to the questionnaire, results from the initial blood draw, and preliminary ultrasound screening may exclude you from the study.

SUBJECT PREPARATION / INCLUSION/EXCLUSION CRITERIA

To participate in the study you must:

- Be of moderate risk described by ACSM guidelines
- Be a male over the age of 45 years old
- Have a Body Mass Index (BMI) greater than 25 kg/m^2
- Be sedentary defined by the surgeon general
- Have permission from your physician to participate in this investigation
- Have fasted for the past 12 hours
- Not have any tobacco products at least 8hrs prior to each treatment day
- Not exercise for at least 12hrs prior to each treatment day
- Not have any caffeine at least 8hrs prior to each treatment day
- Not have any vitamin supplementation for at least 8hrs prior to each treatment day
- Not have any trace of medications that dilate your arteries in your bodies system

- If you are on any medications that dilate your arteries and/or cannot be tested within the preparation criteria listed above you will be excluded from the study

You will be excluded from the study if you:

- Are not 45 years of age or older,
- Do not fall into the moderate risk classification (ACSM),
- Cannot exercise at 75% VO_2peak for 45 minutes,
- Have a known allergy to heparin,
- Do not have peripheral venous access in the left upper extremity
- Have cardiovascular, pulmonary or metabolic diseases
- Have orthopedic problems that limit your exercise
- Take medications that dilate your arteries such as nitroglycerin or verapamil
- Take Aspirin and cannot discontinue use 3 days prior to each treatment
- Take any non-steroidal anti-inflammatory and cannot discontinue use 1 day prior to each treatment

RISK STRATIFICATION FORM

The risk stratification form summarizes information about your medical history, present diseases/disorders, family history of disease, height and weight, and current medications along with information that will be obtained from your fasting blood draw. If there is any question regarding any information presented in the risk stratification form, your physician may be consulted.

FASTING BLOOD DRAW

This test is to determine your risk stratification for exercise. You will report to the Indiana University Health Center (Room 208). You must be fasting for at least 12 hours prior to this test. A 20-45 ml sample of venous blood will be drawn by a certified technician via sterile techniques for analysis of cholesterol, triglycerides and glucose. This test will take approximately 10-15 minutes.

MAXIMAL GRADED EXERCISE TEST

This test is designed to measure your exercise capacity. In the presence of a physician, you will walk or jog on a motor-driven treadmill beginning at a speed between 2.5-5.0 mph, 0% grade, with the grade or slope increasing 2.5% every two minutes until volitional fatigue, breathlessness, chest discomfort, and/or any other symptoms which indicate to yourself or the technicians that you should stop exercise. Heart rate, 12-lead EKG will be monitored throughout the test. Blood pressure will be taken before, during if walking (every stage), and after the test (recovery). Expired gases will also be collected through the mouthpiece during the test to measure fitness and identify the appropriate exercise intensity. The total duration of this test is approximately 45 minutes. There is a one hour recovery period following maximal exercise testing during which you should not subject yourself to additional stress such as a very hot or very cold shower, smoking, heavy food intake, or other exercise.

ARTERIAL HEALTH or FLOW MEDIATED DILATION (FMD)

How well your artery expands with changes in blood flow is used to characterize the health of your arteries. Three EKG electrodes will be positioned on your chest. Following EKG placement, you will be instructed to lie on your back (connected to the EKG machine) in a dark, climate controlled room (22-24°C or 72-75 F) with your right arm extended laterally to establish a resting state. After the 20 minute resting phase, a water based silicon gel will be placed on the ultrasound transducer as an impedance adapter for better ultrasound images. The Sonoace Pico ultra sound system using a 7.0 MHz linear transducer will then be used to scan the resting artery of your inner arm, 2-10 cm above your elbow. Once a clear image is obtained, resting blood flow velocity and a baseline image of your artery will be captured by the ultrasound. After baseline measurements are captured, a blood flow cuff will be wrapped around the forearm of the right arm and inflated to 250 mm Hg to stop blood flow to your lower arm for 5 minutes. Blood flow velocity will be recorded for 10 seconds after the cuff is deflated and ultrasound images will be captured as described above for the remaining duration (~2 minutes). The health of your artery will be expressed as a percentage of the artery expansion with the increased blood flow, compared to the resting diameter.

EXERCISE INTERVENTIONS (25%, 50%, and 75% of Exercise Capacity)

You will be asked to perform three separate walking exercise sessions at 25%, 50%, and 75% of your exercise capacity (on three separate days) obtained from the maximal graded exercise test. Following this single bout of exercise, FMD will be performed immediately and at one, two and three hours post exercise.

Expired gases will be measured through a mouthpiece during the 5^{th} minute of each exercise session to confirm your exercise intensity. The work rate will be adjusted if it is not within ±10% of your target exercise intensity. Expired gases will then be measured again during the 15^{th} minute of exercise to confirm the new exercise intensity. Heart rate and blood pressure will be monitored throughout each exercise session.

SERIAL BLOOD DRAWS via ANGIOCATH

At the beginning of the study day, a 22-24 gauge venous catheter will be inserted in a vein in your left arm by a trained exercise physiologist. The catheter will remain in your arm for the entire duration of the testing day (approximately 6-8 hrs). For each measurement of FMD performed during the study, the catheter will be flushed with normal saline, and a small blood sample (10-20ml) will be collected from the inserted catheter and stored for the analysis of TNF-alpha and IL-6; biomarkers linked to inflammation and arterial health. Upon completion of the testing day, the trained exercise physiologist will remove the catheter from your arm.

RISKS

The discomforts and potential risks associated with this study are minimal.

Risk Stratification Form: There are no risks associated with the questionnaire.

Fasting Venous Blood Draw: The risks associated with this test may include fainting, soreness, bruising, and/or swelling at the venipuncture site. This risk is minimized by having the blood drawn by a trained technician while you are in a seated position at the IU Health Center.

Maximal Graded Exercise Test: The discomforts involved with maximal graded exercise testing can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats and abnormal blood pressure responses. The risk of a serious event, although rare (sudden death = 1/10,000; heart attack = 4/10,000 tests; and complications requiring hospitalization = 10/10,000) does exist. Reasonable effort will be made to conduct the test in such a way as to minimize your discomfort and risk. The mouthpiece will be sanitized with the proper detergents. In the unlikely event of an emergency we will call Bloomington Hospital Ambulance Service. Our laboratory is equipped to respond to certain situations and our personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.

Flow-Mediated Dilation: The risks associated with the placement of **EKG** electrodes may include redness and/or itching at the electrode sites. The risks associated with forearm occlusion when measuring **FMD** may include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with **ultrasound measurements** may include skin irritation and pressure around the transducer sites. The risks associated with the **Ultrasound lubricant gel** are skin irritation and possible break out of rash.

Walking Exercise Intervention: The discomforts involved with exercise sessions can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats, and abnormal blood pressure responses. The risk of an event, although rare, is significantly reduced from exercise testing because of the sub-maximal intensity (morbidity = 1/887,526 participant hours of exercise and mortality = 1/1,124,000 participant hours). Reasonable effort will be made to minimize risk through prior screening and the use of proper warm-up, exercise and cool-down technique. The mouthpiece will be sanitized with the proper detergents. In the unlikely event of a heart related emergency we will call the Bloomington Hospital Ambulance Service. The laboratory is equipped to respond to such situations and its personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.

Continuous Blood Draw via Angiocath: The risks associated with the blood draw via angiocath may include infection, allergic reaction to heparin, irritation and bruising of the skin, pain, discomfort, collapsed vein, multiple puncture sites, and fainting. This risk is minimized by having the catheter inserted in a supine or seated position. If a mild reaction (ie. local swelling or redness at site) to heparin occurs, oral Benadryl® will be provided. If a serious reaction (ie. rash, swelling of the face, anaphylaxis) the emergency protocol will be initiated. This risk, although rare, is minimized by using proper sterile technique. There is a chance of phlebitis which can cause redness, swelling, moderate

discomfort, and fever for up to a few days after the catheter is removed. If mild symptoms of phlebitis develop, warm compresses, alternating Tylenol® and Advil® will be indicated till the symptoms are gone (usually 12-72 hours). If serious symptoms of phlebitis develop (ie. fever or red streaks up the arm) the subject will be instructed to seek medical care immediately. This risk is minimized by using proper sterile technique.

Every effort will be made to minimize these risks.

BENEFITS

This new non-invasive technique to measure the health of the artery may provide further insight on the development and treatment of disease. Using exercise as a non-pharmacological intervention may create a safer and more effective treatment in prevention and management of disease. This study is one of many that will aid in discovering the most efficient exercise treatment in disease. You will receive information on your maximal exercise capacity, fasting blood work, and most importantly how well your arteries respond to different intensities of single session exercise.

CONFIDENTIALITY

All laboratory data will be collected in the privacy of the Clinical Exercise Physiology Laboratory. All data will be coded and filed with the reference to the subjects' participant number. Codes are stored in the graduate office of the clinical exercise physiology lab in a card file separate from the data files. All data will be stored in files locked in the Clinical Exercise Physiology Laboratory. Any part of the results obtained throughout this investigation will be made available to your physician if you desire. No subject will be referred to by name in any publication or summary of this work. Data collected will be stored and kept by your research participation number and may be used for future research studies. These data will be kept indefinitely because they contain medical related information. You are free to withdraw from the study at any time.

CONTACT

If you have any questions at any time regarding this study or the procedures, (or you experience adverse effects as a result of participating in the study) you may contact:

Ryan Harris	HPER 070	Indiana University, Bloomington, IN 47405 - 855-7556
Dr Wallace	HPER 112G	Indiana University, Bloomington, IN 47405 - 855-6384
Dr Rink	HPER 070	Indiana University, Bloomington, IN 47405 - 855-7556

If you feel that you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact the office for Human Subjects Committee, Indiana University, Carmichael Center L03, 530E. Kirkwood Ave., Bloomington, IN 47408 or call 812-855-3067, email: iub_hsc@indiana.edu.

PARTICIPATION

You are free to decline to answer specific items or questions in interviews or on questionnaires, and are free to withdraw from the study at any time. If you do withdraw from the study before data collection is completed, your data will be returned to you or destroyed upon your request.

PARTICIPATION IN RELIABILITY TEST (check one)

Approximately 10 subjects are needed to repeat the 50% exercise intensity treatment day to evaluate reproducibility of the results. Willingness to participate in this part of the study will be solicited during initial consent.

_____ I am interested in repeating one treatment as part of a reliability test.

_____ I am not interested in repeating one treatment as part of a reliability test.

CONSENT

I certify to the best of my knowledge and belief, I have no physical or mental illness or weakness that would increase my risk of participation in this study.

I have read this form and received a copy of it. I have had all my questions answered to my satisfaction. I agree to take part in this study.

Subjects Signature_____ Date_____

Investigators Signature_____ Date_____

Consent Form Updated: July 26, 2006

INFORMED CONSENT (Active)

INDIANA UNIVERSITY - BLOOMINGTON
Informed Consent Statement

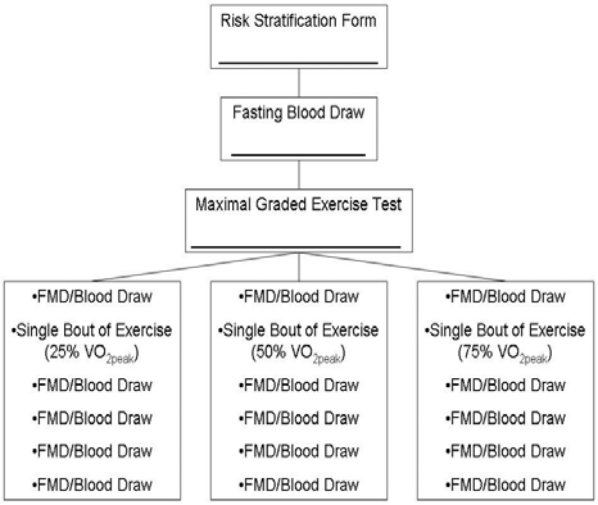
The Interaction of TNF-α, IL-6, and Acute Exercise on Endothelial Function

You are invited to participate in a research project under the supervision of Janet P. Wallace, Ph.D., Ryan Harris, M.S., and Larry Rink, M.D., at Indiana University. The purpose of this study is a) to investigate how the exercise intensity is related to the health of your arteries, and b) to investigate whether the exercise effects are related to the biomarker TNF-alpha, a marker of inflammation.

RESEARCH DESIGN INFORMATION

There will be approximately 15-20 subjects recruited for this investigation. Participation of this study will required you to 1) fill out a risk stratification form, 2) have fasting blood taken at the IU health center, and 3) perform a maximal graded exercise test on a separate day to determine the appropriate exercise intensity (25%, 50%, or 75% VO_2peak).

Flow-Mediated Dilation and TNF-alpha will be taken at baseline and post exercise sessions. Each subject will take approximately 2-3 weeks for completion of this study.



Note: Some answers to the questionnaire, results from the initial blood draw, and preliminary ultrasound screening may exclude you from the study.

SUBJECT PREPARATION / INCLUSION/EXCLUSION CRITERIA

To participate in the study you must:

- Be of moderate risk described by ACSM guidelines
- Be a male over the age of 45 years old
- Have a Body Mass Index (BMI) more than 25 kg/m^2
- Be active defined by the surgeon general
- Have permission from your physician to participate in this investigation
- Have fasted for the past 12 hours
- Not have any tobacco products at least 8hrs prior to each treatment day
- Not exercise for at least 12hrs prior to each treatment day
- Not have any caffeine at least 8hrs prior to each treatment day
- Not have any vitamin supplementation for at least 8hrs prior to each treatment day
- Not have any trace of medications that dilate your arteries in your bodies system

- If you are on any medications that dilate your arteries and/or cannot be tested within the preparation criteria listed above you will be excluded from the study

You will be excluded from the study if you:

- Are not 45 years of age or older,
- Do not fall into the moderate risk classification (ACSM),
- Cannot exercise at 75% VO_2peak for 45 minutes,
- Have a known allergy to heparin,
- Do not have peripheral venous access in the left upper extremity
- Have cardiovascular, pulmonary or metabolic diseases
- Have orthopedic problems that limit your exercise
- Take medications that dilate your arteries such as nitroglycerin or verapamil
- Take Aspirin and cannot discontinue use 3 days prior to each treatment
- Take any non-steroidal anti-inflammatory and cannot discontinue use 1 day prior to each treatment

RISK STRATIFICATION FORM

The risk stratification form summarizes information about your medical history, present diseases/disorders, family history of disease, height and weight, and current medications along with information that will be obtained from your fasting blood draw. If there is any question regarding any information presented in the risk stratification form, your physician may be consulted.

FASTING BLOOD DRAW

This test is to determine your risk stratification for exercise. You will report to the Indiana University Health Center (Room 208). You must be fasting for at least 12 hours prior to this test. A 20-45 ml sample of venous blood will be drawn by a certified technician via sterile techniques for analysis of cholesterol, triglycerides and glucose. This test will take approximately 10-15 minutes.

MAXIMAL GRADED EXERCISE TEST

This test is designed to measure your exercise capacity. In the presence of a physician, you will walk or jog on a motor-driven treadmill beginning at a speed between 2.5-5.0 mph, 0% grade, with the grade or slope increasing 2.5% every two minutes until volitional fatigue, breathlessness, chest discomfort, and/or any other symptoms which indicate to yourself or the technicians that you should stop exercise. Heart rate, 12-lead EKG will be monitored throughout the test. Blood pressure will be taken before, during if walking (every stage), and after the test (recovery). Expired gases will also be collected through the mouthpiece during the test to measure fitness and identify the appropriate exercise intensity. The total duration of this test is approximately 45 minutes. There is a one hour recovery period following maximal exercise testing during which you should not subject yourself to additional stress such as a very hot or very cold shower, smoking, heavy food intake, or other exercise.

ARTERIAL HEALTH or FLOW MEDIATED DILATION (FMD)

How well your artery expands with changes in blood flow is used to characterize the health of your arteries. Three EKG electrodes will be positioned on your chest. Following EKG placement, you will be instructed to lie on your back (connected to the EKG machine) in a dark, climate controlled room (22-24°C or 72-75 F) with your right arm extended laterally to establish a resting state. After the 20 minute resting phase, a water based silicon gel will be placed on the ultrasound transducer as an impedance adapter for better ultrasound images. The Sonoace Pico ultra sound system using a 7.0 MHz linear transducer will then be used to scan the resting artery of your inner arm, 2-10 cm above your elbow. Once a clear image is obtained, resting blood flow velocity and a baseline image of your artery will be captured by the ultrasound. After baseline measurements are captured, a blood flow cuff will be wrapped around the forearm of the right arm and inflated to 250 mm Hg to stop blood flow to your lower arm for 5 minutes. Blood flow velocity will be recorded for 10 seconds after the cuff is deflated and ultrasound images will be captured as described above for the remaining duration (~2 minutes). The health of your artery will be expressed as a percentage of the artery expansion with the increased blood flow, compared to the resting diameter.

EXERCISE INTERVENTIONS (25%, 50%, and 75% of Exercise Capacity)

You will be asked to perform three separate walking exercise sessions at 25%, 50%, and 75% of your exercise capacity (on three separate days) obtained from the maximal graded exercise test. Following this single bout of exercise, FMD will be performed immediately and at one, two and three hours post exercise.

Expired gases will be measured through a mouthpiece during the 5^{th} minute of each exercise session to confirm your exercise intensity. The work rate will be adjusted if it is not within ±10% of your target exercise intensity. Expired gases will then be measured again during the 15^{th} minute of exercise to confirm the new exercise intensity. Heart rate and blood pressure will be monitored throughout each exercise session.

SERIAL BLOOD DRAWS via ANGIOCATH

At the beginning of the study day, a 22-24 gauge venous catheter will be inserted in a vein in your left arm by a trained exercise physiologist. The catheter will remain in your arm for the entire duration of the testing day (approximately 6-8 hrs). For each measurement of FMD performed during the study, the catheter will be flushed with normal saline, and a small blood sample (10-20ml) will be collected from the inserted catheter and stored for the analysis of TNF-alpha and IL-6; biomarkers linked to inflammation and arterial health. Upon completion of the testing day, the trained exercise physiologist will remove the catheter from your arm.

RISKS

The discomforts and potential risks associated with this study are minimal.

Risk Stratification Form: There are no risks associated with the questionnaire.

Fasting Venous Blood Draw: The risks associated with this test may include fainting, soreness, bruising, and/or swelling at the venipuncture site. This risk is minimized by having the blood drawn by a trained technician while you are in a seated position at the IU Health Center.

Maximal Graded Exercise Test: The discomforts involved with maximal graded exercise testing can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats and abnormal blood pressure responses. The risk of a serious event, although rare (sudden death = 1/10,000; heart attack = 4/10,000 tests; and complications requiring hospitalization = 10/10,000) does exist. Reasonable effort will be made to conduct the test in such a way as to minimize your discomfort and risk. The mouthpiece will be sanitized with the proper detergents. In the unlikely event of an emergency we will call Bloomington Hospital Ambulance Service. Our laboratory is equipped to respond to certain situations and our personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.

Flow-Mediated Dilation: The risks associated with the placement of **EKG** electrodes may include redness and/or itching at the electrode sites. The risks associated with forearm occlusion when measuring **FMD** may include redness of the skin, bruising, numbness, pain, tingling of the fingers and discomfort while the cuff is inflated. The risks associated with **ultrasound measurements** may include skin irritation and pressure around the transducer sites. The risks associated with the **Ultrasound lubricant gel** are skin irritation and possible break out of rash.

Walking Exercise Intervention: The discomforts involved with exercise sessions can include episodes of transient light headaches, chest discomfort, leg cramps, occasional irregular heart beats, and abnormal blood pressure responses. The risk of an event, although rare, is significantly reduced from exercise testing because of the sub-maximal intensity (morbidity = 1/887,526 participant hours of exercise and mortality = 1/1,124,000 participant hours). Reasonable effort will be made to minimize risk through prior screening and the use of proper warm-up, exercise and cool-down technique. The mouthpiece will be sanitized with the proper detergents. In the unlikely event of a heart related emergency we will call the Bloomington Hospital Ambulance Service. The laboratory is equipped to respond to such situations and its personnel are trained to administer emergency care in the form of Basic Life Support. Advanced Cardiac Life Support will be provided by Bloomington Hospital Ambulance Service.

Continuous Blood Draw via Angiocath: The risks associated with the blood draw via angiocath may include infection, allergic reaction to heparin, irritation and bruising of the skin, pain, discomfort, collapsed vein, multiple puncture sites, and fainting. This risk is minimized by having the catheter inserted in a supine or seated position. If a mild reaction (ie. local swelling or redness at site) to heparin occurs, oral Benadryl® will be provided. If a serious reaction (ie. rash, swelling of the face, anaphylaxis) the emergency protocol will be initiated. This risk, although rare, is minimized by using proper sterile technique. There is a chance of phlebitis which can cause redness, swelling, moderate discomfort, and fever for up to a few days after the catheter is removed. If mild

symptoms of phlebitis develop, warm compresses, alternating Tylenol® and Advil® will be indicated till the symptoms are gone (usually 12-72 hours). If serious symptoms of phlebitis develop (ie. fever or red streaks up the arm) the subject will be instructed to seek medical care immediately. This risk is minimized by using proper sterile technique.

Every effort will be made to minimize these risks.

BENEFITS

This new non-invasive technique to measure the health of the artery may provide further insight on the development and treatment of disease. Using exercise as a non-pharmacological intervention may create a safer and more effective treatment in prevention and management of disease. This study is one of many that will aid in discovering the most efficient exercise treatment in disease. You will receive information on your maximal exercise capacity, fasting blood work, and most importantly how well your arteries respond to different intensities of single session exercise.

CONFIDENTIALITY

All laboratory data will be collected in the privacy of the Clinical Exercise Physiology Laboratory. All data will be coded and filed with the reference to the subjects' participant number. Codes are stored in the graduate office of the clinical exercise physiology lab in a card file separate from the data files. All data will be stored in files locked in the Clinical Exercise Physiology Laboratory. Any part of the results obtained throughout this investigation will be made available to your physician if you desire. No subject will be referred to by name in any publication or summary of this work. Data collected will be stored and kept by your research participation number and may be used for future research studies. These data will be kept indefinitely because they contain medical related information. You are free to withdraw from the study at any time.

CONTACT

If you have any questions at any time regarding this study or the procedures, (or you experience adverse effects as a result of participating in the study) you may contact:

Ryan Harris	HPER 070	Indiana University, Bloomington, IN 47405 - 855-7556
Dr Wallace	HPER 112G	Indiana University, Bloomington, IN 47405 - 855-6384
Dr Rink	HPER 070	Indiana University, Bloomington, IN 47405 - 855-7556

If you feel that you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact the office for Human Subjects Committee, Indiana University, Carmichael Center L03, 530E. Kirkwood Ave., Bloomington, IN 47408 or call 812-855-3067, email: iub_hsc@indiana.edu.

PARTICIPATION

You are free to decline to answer specific items or questions in interviews or on questionnaires, and are free to withdraw from the study at any time. If you do withdraw from the study before data collection is completed, your data will be returned to you or destroyed upon your request.

PARTICIPATION IN RELIABILITY TEST (check one)

Approximately 10 subjects are needed to repeat the 50% exercise intensity treatment day to evaluate reproducibility of the results. Willingness to participate in this part of the study will be solicited during initial consent.

_____ I am interested in repeating one treatment as part of a reliability test.

_____ I am not interested in repeating one treatment as part of a reliability test.

CONSENT

I certify to the best of my knowledge and belief, I have no physical or mental illness or weakness that would increase my risk of participation in this study.

I have read this form and received a copy of it. I have had all my questions answered to my satisfaction. I agree to take part in this study.

Subjects Signature_____ Date_____

Investigators Signature_____ Date_____

Consent Form Updated: August 28, 2006

HUMAN SUBJECTS AMMENDMENT 4/5/2006

This study amendment is being submitted to modify:

- The number of subjects being recruited for this investigation
- Additional inclusion/exclusion criteria for this investigation
- An additional arterial health measurement and subsequent blood draw
- And, the analysis of another biomarker.

After analysis of the literature and pilot work from our laboratory, the required subjects needed to find significance will be approximately 15-20. This modification does not affect the risk: benefit for the subjects being investigated.

In order to control for certain parameters in measuring arterial health and the biomarkers, more strict criteria for including and excluding subject will be implemented. The complete list of inclusion/exclusion criteria is highlighted in the attached informed consent. This modification does not affect the risk: benefit for the subjects being investigated and will hopefully create more reliable data.

One additional measurement of arterial health and subsequent blood draw will be added immediately following exercise. This additional blood collection (10-20ml) is still within appropriate collection amounts within the investigation time studied. This modification does not affect the risk: benefit for the subjects being investigated and will provide further insight to answering the scientific question being asked.

Following collection and storage of the blood, an additional biomarker will be analyzed. This modification does not affect the risk: benefit for the subjects being investigated.

HUMAN SUBJECTS AMMENDMENT 4/17/2006

This study amendment is being submitted to modify:

- The procedure utilized to measure endothelial function.

The procedure will be similar as previously described, yet include the following:

- Placement and connection of 3 EKG electrodes during the Flow-mediated Dilation procedure.
- Utilization of a specific blood flow cuff as a replacement for the standard blood pressure cuff used to occlude the artery.

The risks associated with the placement of EKG electrodes may include redness and/or itching at the electrode sites; however, these modifications do not affect the overall risk: benefit for the subjects being investigated.

HUMAN SUBJECTS AMMENDMENT 5/5/2006

This study amendment is being submitted to modify:

- The procedure utilized to measure endothelial function.

The procedure will be similar as previously described, yet include the following:

- Placement and connection of 3 EKG electrodes during the Flow-mediated Dilation procedure (FMD).
- Utilization of a specific blood flow cuff as a replacement for the standard blood pressure cuff used to occlude the artery.

The benefit of incorporating EKG will decrease the subjective error associated with the FMD procedure.

The risks associated with the placement of EKG electrodes may include redness and/or itching at the electrode sites; however, these modifications do not affect the overall risk: benefit for the subjects being investigated.

HUMAN SUBJECTS AMMENDMENT 5/17/2006

This study amendment is being submitted to modify:

- The inclusion criteria for the moderate risk population being investigated, and
- To include the option of repeating one of the treatment days to evaluate reproducibility.

The modification to the inclusion criteria is being done to identify a more specific moderate risk population. The inclusion criteria being added are:

- Be a male over the age of 45 years old
- Have a Body Mass Index (BMI) greater than 25 kg/m^2
- Be sedentary defined by the surgeon general

The repetition of one treatment day will help answer an important research question that will be necessary to incorporate for external funding opportunities.

The benefit of identifying a more specific subject population will help when inferring the results to the specific group being investigated. A more diverse subject population may introduce too many demographic confounding variables.

There are no risks associated with the new addition of the inclusion criteria and there are no additional risks associated with participating in an extra treatment day.

HUMAN SUBJECTS AMMENDMENT 6/2/2006

This study amendment is being submitted to modify the statement regarding prostacyclin inhibitors to be more specific and the addition of a Public Service Announcement:

- Alternative pharmacology that needs to be controlled for throughout the duration of the study

- The modification to exclude the use of aspirin and non-steroidal anti inflammatory agents (ibuprofen, Advil, etc.) for the days prior to the treatments will be implemented to control for vascular and inflammatory effects.

- The PSA is attached and will be used to aid in subject recruitment

There are no risks associated with the new addition of the inclusion criteria, as these agents used in the studied population are for prophylactic affects and will be approved by each subject's physician if discontinued use is necessary.

HUMAN SUBJECTS AMMENDMENT 7/26/2006

This study amendment is being submitted to modify the inclusion criteria to include:

- Have fasted for the past 12 hours

There are no risks associated with the new addition of the inclusion criteria. To control for a meal effect on arterial health, subjects need to be fasting.

HUMAN SUBJECTS AMMENDMENT 8/28/2006

This study amendment is being submitted to modify the population being investigated in addition to the overweight/sedentary group currently being investigated. The new population will include:

- Overweight/active Men

There are no risks associated with the new addition of the inclusion criteria.

APPENDIX F – BIOHAZARD SAFETY REVIEW AND APPROVAL

BIOHAZARD SAFETY APPROVAL

Study No. 05-018

INSTITUTIONAL BIOSAFETY REPORTING AND APPROVAL FORM
Indiana University

This form must be submitted for **ALL** activities (e.g., research, teaching) involving recombinant DNA (rDNA), infectious agents, TAT proteins, toxins, prions, and/or human tissue and fluids use.

Submit the original and two copies to: Beth Reeves, Biosafety Officer, Environmental Health and Safety, 2735 E 10th St Room 160, Indiana University 47408. Call 855-9333, or e-mail bereeves@indiana.edu, for additional forms and information. (Keep a copy for your records.)

I. CAMPUS Bloomington SCHOOL/DEPARTMENT Kinesiology

PRIN. INVEST/INSTR Janet P. Wallace, Ph.D.
ID# (IU ID# or if you choose your S.S.#) 2 0000 001 019 179
CO-PRIN.INVEST/INSTR Julie Frey, M.S.
ID# (IU ID# or if you choose your S.S.#) 0000 881937
CO-PRIN.INVEST/INSTR Alyce Fly, Ph.D.
ID# (IU ID# or if you choose your S.S.#) 361-44-6085
CO-PRIN.INVEST/INSTR Larry Rink, M.D.
ID# (IU ID# or if you choose your S.S.#) 314-42-2953
CO-PRIN.INVEST/INSTR Tim Mickleborough, Ph.D.
ID# (IU ID# or if you choose your S.S.#) 2 0593 899 857 553

Campus Address: HPER 112-G Office Phone: 5-6384
Lab Phone: 5-5776 Home Phone: 334-8217
E-mail address: wallacej@indiana.edu
RANK: Faculty X Res. Scientist Post-Doc Staff

Student Ryan Harris ID# 0001860960 Faculty advisor Janet P. Wallace

Citizenship: PI USA Co-PI USA Student(s) USA

PROJECT TITLE: Can exercise and physical activity counteract the effects of a high fat meal on endothelial function?

BIOHAZARD SAFETY PROPOSAL

Study No.

INSTITUTIONAL BIOSAFETY REPORTING AND APPROVAL FORM
Indiana University

This form must be submitted for **ALL** activities (e.g., research, teaching) involving recombinant DNA (rDNA), infectious agents, TAT proteins, toxins, prions, and/or human tissue and fluids use.

Submit the original and two copies to: Beth Reeves, Biosafety Officer, Environmental Health and Safety, 2735 E 10th St Room 160, Indiana University 47408. Call 855-9333, or e-mail bereeves@indiana.edu, for additional forms and information. (Keep a copy for your records.)

I. CAMPUS Bloomington

SCHOOL/DEPARTMENT Kinesiology

PRIN. INVEST/INSTR Janet P. Wallace, Ph.D.

ID# (IU ID# or if you choose your S.S.#) 2 0000 001 019 179

CO-PRIN.INVEST/INSTR Julie Frey, M.S.

ID# (IU ID# or if you choose your S.S.#) 0000 881937

CO-PRIN.INVEST/INSTR Alyce Fly, Ph.D.

ID# (IU ID# or if you choose your S.S.#) 361-44-6085

CO-PRIN.INVEST/INSTR Larry Rink, M.D.

ID# (IU ID# or if you choose your S.S.#) 314-42-2953

CO-PRIN.INVEST/INSTR Tim Mickleborough, Ph.D.

ID# (IU ID# or if you choose your S.S.#) 2 0593 899 857 553

Campus Address: HPER 112-G Office Phone: 5-6384

Lab Phone: 5-5776 Home Phone: 334-8217

E-mail address: wallacej@indiana.edu

RANK: Faculty x Res. Scientist Post-Doc Staff

Student Ryan Harris ID# 0001860960 Faculty advisor Janet P. Wallace

Citizenship: PI USA Co-PI USA Student(s) USA

PROJECT TITLE: Can exercise and physical activity counteract the effects of a high fat meal on endothelial function?

TITLE OF GRANT UNDER WHICH PROJECT WILL BE CONDUCTED (if different than project title)

1. American Heart Association: Can exercise and physical activity counteract the effects of a high fat meal on endothelial function?
2. American Diabetes Association: Can exercise and physical activity counteract the effects of a high fat meal on endothelial function in diabetes?
3. NIH: Can exercise and physical activity counteract the effects of a high fat meal on endothelial function in obesity?

If this activity involves other campuses, please indicate which ones:

FUNDING SOURCE AHA, ADA, NIH **APPLICATION DEADLINE** Jan & Feb 2006

Proposed Start Date: July 06; pilot data Fall 05 or renewal date

PROJECT TYPE: Research _x_ Project#_____New_____Renewal
Teaching__x_ Course #_K602__New____Renewal
Teaching is for the pilot work; supervised by Drs. Fly & Mickleborough and Julie Frey

LOCATION: List the building and room number of each laboratory in which any part of the experiments will be conducted HPER Rooms 070 (collection) and 005 & 078 (analysis & storage)

If grant proposal, list grant numbers and all agencies _1) American Heart Association (January 2006), 2) American Diabetes Association (January 2006), and 3) NHLBI of NIH (February 2006)__

TERM OF PROJECT: START_Fall 2005____ **END**__June 2011_____

If this application requires BL-2 or higher containment circle "yes" or "no":

YES **NO** The facilities used in these activities have been previously inspected by the IBC and meet appropriate biological laboratory safety standards. 005 (Dr. Fly), 070 (Dr. Wallace) and 078 (Dr. Mickleborough) have been previously approved for biosafety standards

Indicate (check) agents or materials used and follow instructions:

_____Recombinant DNA. Fill out sections even if exempt: Complete sections A,B,C & D as required.

_____Infectious agents, toxins, TAT proteins, or prions (pathogenic to humans, animals or plants) Fill out section E

__x__Human tissues or fluids. Fill out section F

_____Use and/or possession of select agents according to the Patriot Act (http://www.indiana.edu/~resrisk/patriotactsummary.pdf). Fill out section G

COMPLETION AND SIGNING OF THIS FORM ARE THE RESPONSIBILITY OF THE PRINCIPAL INVESTIGATOR OR FACULTY MEMBER IN CHARGE.

In signing this form, I agree to abide by all university and federal guidelines and regulations regarding recombinant DNA, infectious agent, TAT protein and/or human tissues and fluids work.

Principal Investigator is responsible for all liabilities related to use of his/her materials.

_____6 October 2005_____

Principal Investigator (and Faculty Advisor) Signature **Date**

A. Experiments that require RAC or ORDA review; NIH and IBC approval. Circle "Yes" or "No":

YES NO 1. Deliberate formation of rDNAs containing genes for biosynthesis of toxic molecules.

YES NO 2. Deliberate release into the environment of any organism containing rDNA.

YES NO 3. Deliberate transfer of drug resistance trait to microorganisms such that drug control might be compromised.

YES NO 4. Deliberate transfer of rDNA into human subjects.

B. Experiments that require IBC approval before initiation. Circle "Yes" or "No":

YES NO 1. Use of other than a Class 1 agent as host-vector system (see Appendix B of NIH Guidelines, 1995).

YES NO 2. Will you use a Class 2,3, or 4 viral vector? If so, will:

(**YES NO**) a. Greater than 2/3's of genome be used?

(**YES NO**) b. helper virus be used?

(**YES NO**) c. your experiment enhance pathogenicity (e.g., insertion of oncogene, extend host range)?

YES NO 3. Will whole animals or plants be used as hosts?

YES NO 4. Will experiments involve more than 10 liters of culture?

YES NO 5. Will a deliberate attempt be made to obtain expression of a foreign gene?
If so, what protein/RNA will be produced?

YES NO 6. Will TAT proteins be purchased, synthesized or expressed?

YES NO 7. Will a toxin be used?

YES NO 8. Will prions be used?

ALL "YES" ANSWERS ABOVE MUST BE EXPLAINED IN SECTION BELOW.

C. rDNA Constructs:

1. Host organism:

2. List the vector(s) name and type (e.g., -gt11, retroviral pLNL), and append a DNA map of any novel vectors listing components with their sizes).

3. Source organism of DNA to be cloned (e.g., human T-Cell cDNA library, HIV gag gene):

4. If the DNA is microbial, circle the appropriate class as given in Appendix B [Current federal guidelines: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm]
   1 2 3 4 5 N/A

5. Function of products (protein or RNA) of the cloned DNA:

6. For oncogenic viruses, circle appropriate level (Appendix B [and all federal guidelines and Biosafety Manual]:

   Low Risk Moderate Risk N/A

7. Circle containment level specified by the current Federal Guidelines (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm)and the Biosafety Manual:

Exempt

BL1

BL2

BL3

BL4

Note: All human tissue/body fluid samples require BL2.

D. Include a succinct description of your project on a separate page and explain WHAT, WHY, and HOW rDNA will be used in the project. This CANNOT be replaced with a grant proposal or reprint.

E. Description of proposed research involving infectious agents, toxins, TAT proteins, or prions.

a. Submit a succinct statement of your project on a separate page. Explain why and how infectious agent(s), toxins, TAT proteins, or prions will be used in the project. This CANNOT **be replaced with a grant proposal or a reprint.**

b. List the infectious agent(s), toxins, TAT proteins, or prions:

c. Circle level of research according to the federal guidelines and Biosafety in Microbiological and Biomedical Laboratories (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).(BL-1, BL-2, BL-3, BL-4)

F. Human Tissues and Fluids Usage. Submit to the IBC a statement of that intent and include an outline (abstract) of the proposed activity, and sufficient information that will clarify to the reader that the guidelines involving human tissue and fluids are understood and that the submitting individual is able and intends to adhere to those guidelines.

The purpose of this study is to determine if prior exercise or physical activity prevents the postprandial attenuation in brachial artery FMD produced by ingestion of a high fat meal and to provide insight into the underlying physiological mechanisms.

The design is illustrated in Figure 1. Three different activity sessions and one control session will be presented before the consumption of a high-fat meal in a randomized cross over design. The duration of the physical activity will be from 7:00 am to noon. The single long session of exercise will begin at 11:20 am and end at noon. The four short 10 minute bouts of exercise will begin at 8:40 am, 9:40 am, 10:40 am and 11:40 am; also ending at noon. FMD will be measured 1) at baseline at 6:30 am, 2) before the high-fat meal, 3) every hour, for five hours following the high fat meal, beginning one hour after the high-fat meal, and 4) at 6:30 am the next day. Oxidative and inflammatory biomarkers that will be measured in our proposed study will include 1) Interleuklin-6 (IL-6), 2) nuclear factor κB (NF ιB), 3) Tumor Necrosis Factor (TNF ∀), and 3) 8-epi-prostaglandin F2∀ (8-PG $F_{2\forall}$). IL-6, NF κB, TNF ∀, and 8-PG $F_{2\forall}$ will be measured following every FMD measurement, except for the 6:00 am next day measurement period.

Time	Control	Long	Short	Activity
6:30 AM	Baseline FMD	Baseline FMD	Baseline FMD	Baseline FMD
6:50	Marker	Marker	Marker	Marker
7:00	Breakfast	Breakfast	Breakfast	Breakfast
7:10				Physical Activity
7:20				⇩
7:30				⇩
7:40				⇩
7:50				⇩
8:00				⇩
8:10				⇩
8:20				⇩
8:30				⇩
8:40			Exercise	⇩
8:50			⇩	⇩
9:00				⇩
9:10				⇩
9:20				⇩
9:30				⇩
9:40			Exercise	⇩
9:50			⇩	⇩
10:00				⇩
10:10				⇩
10:20				⇩
10:30				⇩
10:40			Exercise	⇩
10:50			⇩	⇩
11:00				⇩
11:10				⇩
11:20		Exercise		⇩
11:30		⇩		⇩
11:40		⇩	Exercise	⇩
11:50		⇩	⇩	⇩
12:00				
1:00	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
1:15	**High-Fat Meal**	**High-Fat Meal**	**High-Fat Meal**	**High-Fat Meal**
2:15	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
3:15	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
4:15	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
5:15	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
6:15	FMD/Marker	FMD/Marker	FMD/Marker	FMD/Marker
6:30	Dinner	Dinner	Dinner	Dinner
6:30 AM	FMD	FMD	FMD	FMD

Figure 1. Study Design. **FMD** indicates the measurement intervals for flow-mediated dilation. **Marker** indicates the measurement interval for

Biomarkers: Seven blood draws and urine samples will be collected; baseline, pre-, and hourly for five hours post the high-fat meal. Each blood sample will be analyzed for concentrations of IL-6, TNF ∀, and NF κB. The urine samples will be analyzed for 8-PG $F_{2\forall}$.

Blood Collection: a 24 gauge venous catheter equipped with a PRN adapter will be inserted in a vein in the subjects left arm by a clinical exercise physiologist who has special IV training and practice. The catheter will remain there for the entire duration of the testing day (approximately 12

hrs). For each measurement, the catheter will be flushed with normal saline, and a small blood sample (10-20 ml) will be collected from the inserted catheter and immediately transferred into ethylendedinaminetetraacetic (EDTA) vacutainer tubes (Vacutainer, Becton and Dickinson, Meylan, France). Any period of time lasting longer than 2 hours between sample collections will require the IV to be flushed with a heparin based solution to prevent clotting of the catheter. Once the testing day is completed, the catheter will be removed by the same trained clinical exercise physiologist.

Insertion of the antiocath and blood collection will be performed in HPER 070K and be taken to the biochemistry lab down the hall (HPER 005 & 078) for preparation, storage and analysis. Sterile techniques, standard microbiological practices, and universal precautions will be practiced at all times. Latex gloves will be worn during the insertion of the antiocath, the collection of blood, during the analysis of blood, and during any clean-up procedures. Needles (re-sheathe with syringe) will be discarded in sharps containers and alcohol wipes, gauze pads, other angiocath supplies as well as blood analysis supplies such as pipette tips tubes, etc will be discarded in biohazard waste bags. Full biohazard waste bags and sharps containers will be disposed of through the Institutional Biosafety and BioHazard Office. Spills, should they occur, will be absorbed with paper towels and discarded in the biohazard waste bags. A 1:10 Chlorox solution (less than 24 hours old) will set in the spill area for 20 minutes. The items used to clean will be discarded in the biohazard waste bags.

On two testing occasions, subjects will be leaving the lab and returning to their daily routine or performing physical activity with the angiocath in place. Upon departure from the CEP laboratory with the angiocath inserted, Tegaderm® (a see through type tape) will be placed over the entire venipuncture area to seal and prevent the IV area from contamination. Coban® will then be wrapped around the arm to ensure stability of the angiocath. If the IV is placed in the hand, the same procedure described above will take place and the Coban® will be used whenever possible (i.e. depending on the site of the needle incertion). Instead, latex gloves will be given to the subject in to cover the access site, in case they want to wash their other hand.

A locked traveling kit1 will be used if any of these subjects report having trouble with their angiocath and are in need of help while they are not in the lab. The clinical exercise physiologist will be paged to respond to any situation. The traveling kit will contain 1) latex gloves, 2) angtiocath kits, 3) sterile drapery, 4) gauze and alcohol wipes, 5) paper towels for cleaning spills, 6) Clorox solution, 7) lab coat, 8) biohazard bags, and 9) sharps container.

Urine Collection: Urine will be collected in sterile urine cups, by the subject in the privacy of the rest room (men's room is HPER 089 and women's room is HPER 087). The sample will be left on the rest room sink for the investigator to pick-up immediately. The sample will be then taken to the biochem labs (HPER 005 & 078) around the corner for preparation. Latex gloves will be worn during the handling and analysis of all urine samples. Used urine cups and related materials will be discarded in biohazard waste bags. Full biohazard waste bags will be disposed of through the Institutional Biosafety and BioHazard Office. Spills, should they occur, will be absorbed in paper towels and discarded in the biohazard waste bags. The spill area will be cleaned with Clorox solution of 1:10 and the items used to clean will be discarded in the biohazard waste bags.

Biosafety Level 2 Precautions: Work with human fluids is considered to be biosafety level 2. Appropriate precautions for Level 2 include:

1. Laboratory coats will be worn.
2. Latex gloves will be worn.
3. Protective eyewear will be worn.
4. Laboratory areas have vinyl flooring
5. Sinks are located in HPER 005, 070K, 078, 087 and 089
6. Bench tops in 070 and 078 are impervious to water and are resistant to moderate heat and organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface
7. Laboratory cot and blood drawing chair are used to insert the angiocath and draw blood. These furniture are capable of supporting subjects and are accessible for cleaning.
8. There are no windows in laboratories in 070K, 078. The windows in HPER 005 open to the exterior and are fitted with fly screens.

---

1 Only the doctoral student (Ryan Harris) who uses the traveling kit and the lab director (Janet P. Wallace) will have keys to the traveling kit.

9. Any time urine or blood samples are transported from one site to another, they will first be placed in a plastic sealed container, labeled with biohazard symbol, outside of container. The container will be disinfected, and the transporter will wash their hands before transport.
10. Standard microbiological practices will be used
    a. Restriction to the laboratories will be made during blood and urine collection and analyses
    b. Laboratory personnel will wash their hands after handling materials, removing gloves, and before leaving the laboratory.
    c. No eating or drinking is allowed in the laboratories. No food will be stored in any refrigerators used to store samples.
    d. Mouth pipetting will not be practiced.
    e. Work surfaces will be decontaminated after each use.
    f. All waste will be sent to Institutional Biosafety and BioHazard Office for disposal.
11. Eyewash stations are in HPER 005, 070E and 078.
12. Laboratory personnel will have the opportunity to receive the appropriate immunizations
13. Laboratory personnel will take the biosafety course each year.
14. Needles and syringes will be restricted to the laboratory 070K and the locked traveling kit.
15. disposable syringes will be used to collect blood samples through the angiocath. Once the blood sample is transferred to the EDTA tube, the needles will be removed and place in the sharps container.
16. Broken glassware will be handled using tongs and wearing latex gloves
17. Spills and accidents that result in overt exposures to infectious materials will be immediately reported to the laboratory director who will report to the Institutional Biosafety and BioHazard Office. Medical evaluation will be made on those who were exposed. Documentation will be kept.

APPENDIX G – SUBJECT RECRUITMENT AND DATA COLLECTION FORMS

PUBLIC SERVICE ANNOUNCEMENT

Are you a Man 45 years of age or older? Do you not exercise as much as you would like?

Indiana University Clinical Exercise Physiology Laboratory seeks volunteers for exercise and arterial health study

The Indiana University Clinical Exercise Physiology Lab is currently seeking Men who are 45 years old or older who don't exercise as much as they would like. The purpose of the study is to investigate three bouts of different intensity exercise and how your arterial health responds to each bout. Each participant will receive free exercise testing, blood work, and exercise prescription. Prior physician approval is required to participate.

For more information on this study # 05-10379 contact Ryan Harris at the Indiana University Clinical Exercise Physiology Laboratory, 812-855-7556 or Harrisra@indiana.edu.

TELEPHONE SCRIPT

Hello, my name is _____ and I am one of the study coordinators of the arterial stiffness and exercise study at Indiana University.

Thank you for your interest in this study.

The purpose of this study is to describe the how your arteries respond for up to six hours following a single bout of exercise, and to investigate if the intensity of the exercise is related to how well your arteries respond and possibly identify a specific biomarker (TNF-alpha) that plays an important role in how your arteries respond.

Are you still interested in participating?

If the answer is NO:

Thank you very much for calling. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

To be eligible for this study you must be between 18 and 60 years of age, and must not:

- Have had a heart attack, lung disease or diabetes
- Have gallbladder disease
- Have bone and joint problems that limit your exercise
- Take medications that dilate your arteries such as nitroglycerin, prostacyclin, or verapamil
- Have an allergy to heparin

Do you meet these criteria?

If the answer is NO:

I am sorry; we need to control for these variables. Thank you for your time. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

To participate, you also need to:

- Not exercise or perform strenuous physical activity at least 12 hours before each testing session.
- Not have any caffeine at least 8 hours before each testing session.

- Not have Vitamin C at least 8 hours before each testing session.
- Not have tobacco products at least 8 hours before each testing session.
- Fast for 8 hours before each of the testing sessions.

Will you be able to follow these instructions?

If the answer is NO:

I am sorry; we need to control for these variables. Thank you for your time. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

You meet all the initial requirements to be eligible for the study.

Are you interested to come into the lab and go over all of the specifics (ie. fasting blood draw, initial risk stratification form, etc)? We need to get some initial information that may determine you are ineligible to participate in the study

If the answer is NO:

Thank you very much for calling. If you know of anyone who might be interested, please give them our phone number.

If the answer is YES:

We need to schedule an appointment to go over the informed consent during which I will be able to describe all the procedures of the study in detail.

[Scheduling part]

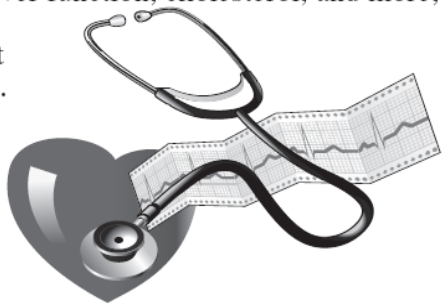
Thank you very much for your time. I look forward to meeting you on that day.

Have a great day!

ARTERY HEALTH STUDY

The IU Clinical Exercise Physiology Lab is currently seeking Men who are 45yo or older to investigate how the arterial health responds to exercise.

THE BENEFITS: Each participant will receive FREE results including: exercise capacity and stress test, fasting blood results of inflammation, liver function, cholesterol, and more, and exercise prescription. Also, each subject will be contributing to validate this non-invasive measurement that will be used in the prevention/treatment of disease. Prior physician approval is required to participate.



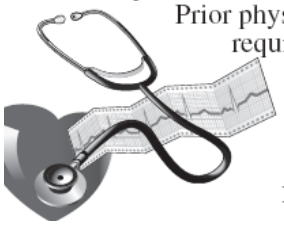
For information on this study # 05-10379
contact Ryan Harris, 812-855-7556 or
Harrisra@indiana.edu.

PARTICIPANTS NEEDED

Participants Needed for Arterial Health and Exercise Study

The IU Clinical Exercise Physiology Lab is currently seeking Men who are 45 y.o. or older. The purpose is to investigate how your arteries respond to exercise.

THE BENEFITS: Each participant will receive FREE results including: exercise capacity and stress test. Fasting blood results of inflammation, liver function, cholesterol and more with exercise prescription. Also, each subject will be contributing to validate this non-invasive measurement that will be used in the prevention/treatment of disease. Prior physician approval is required to participate.



For information on this study # 05-10379 contact Ryan Harris, 812-855-7556 or Harrisra@indiana.edu.

HUMAN SUBJECTS RECRUITMENT FLYER

IU HPER
INDIANA UNIVERSITY
KINESIOLOGY
Study # 05-10379

Volunteers Needed for Artery Health Study

Indiana University Clinical Exercise Physiology Laboratory

There are two phases to this study.

The purpose of this investigation is 1) to describe how much/long your arteries respond to a single exercise session, and 2) to investigate how the exercise intensity is related to your arterial health.

Eligible Criteria

You must :

- Be able to exercise for 45 minutes
- Not have had a heart attack, lung disease, diabetes or gallbladder disease
- Not have any bone or joint problems
- Not take medications that dilate your arteries
- Not have any allergies to heparin

Testing Procedures

- Screening Blood Draw
- Ultrasonic Measure of the Health of your Arteries (Phase 1&2)
- Single Exercise sessions (Phase 1&2)
- Continuous Blood Draws (Phase 2 only)

Benefits

- These measurements can be useful to identify potential risk factors for cardiovascular disease
- You will receive all information regarding the health of your arteries and your standard laboratory blood values

You must be a man over the age of 45

For more information Contact:

Ryan Harris, MS
Clinical Exercise Physiology, HPER 070
Phone: 812.855.7556
E-mail: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

Artery Health Study 05-10379
Tel. 812.855.7556
Email: Harrisra@indiana.edu

MEDICAL RELEASE FORM

Physical Examination and Medical History

Name of Patient_____ DOB_____

Medical History

Does the above patient have or ever had the following conditions? (Please check the appropriate column).	No	Yes	Comments
Acute myocardial infarction			
Angina (please comment)			
Congestive Heart Failure (CHF)			
Cardiomyopathy (IHSS) or Valvular heart disease (specify)			
Suspected or known aneurysm			
Embolism or thrombophlebitis			
ECG abnormalities (specify type)			
Active or suspected myocarditis or pericarditis			
Serious systemic disorder (ie: Mononucleosis, Hepatitis)			
Pulmonary disorders (ie: COPD, asthma, hypertension)			
Diabetes (specify type and severity)			
Uncontrolled metabolic disease (ie: Myxedema, Thyrotoxicosis)			
Electrolyte abnormalities (specify type)			
Prescribed medications (specify type)			
Neuromuscular, musculoskeletal, or rheumatoid disorders which would make exercise difficult			
Significant emotional distress			

Physical Examination

Blood Pressure_____mmHg

Heart Sounds (please check if present):

Lifts_____ Heaves_____Clicks_____ Thrills_____Murmurs_____(location

Lung Sounds (please describe auscultation):

Clear_____ Wheezes_____ Rales_____

Quality of peripheral pulses (please check):

Absent_____1/4_____ 2/4_____ 3/4_____ 4/4_____

Edema (please quantify if present):_____

To your knowledge, can this individual perform an exercise stress test? ***If no, please comment.**_____	**Yes**	**No**
This individual is capable of participating in a maximal_____or moderate_____exercise program under the guidance of a certified exercise specialist.		

Date of Health Examination_____ (must be valid within the past six month)

Signed_____M.D._____Date_____

HIPPA AUTHORIZATION FORM

AUTHORIZATION FOR THE RELEASE OF HEALTH INFORMATION FOR RESEARCH

_____

(Name) (Date of Birth)

This authorization relates to:

Project: The interaction of TNF-a, IL-6, and Endothelial Function on Acute Exercise Intensity
IRB Protocol #: 05-10379
Principal Investigator: Ryan Harris, MS

I understand that the law generally protects my medical records and other health information from use without my permission. The type of information that is protected includes: my address and phone number, laboratory tests, medical history, and X-rays reports.

I understand that if I wish to take part in the research project name(s) above, the researchers will need to see the results of any physical examination(s) conducted for me by my doctor(s) and/or any clinic(s) or hospital(s).

Therefore, to allow me to participate in this research study, I give permission for _____ [name the doctor(s), clinic(s), and/or hospital(s)] to release the results of all physical examinations they have conducted on or about me, to:

1. The Indiana University CEP research team, headed by Janet P. Wallace, PhD,
2. The Medical Director, Dr. Larry Rink,

By signing this authorization, I understand and agree that I am giving permission for protected health information indicated above to be used for the purposes of conducting the above study.

I understand that the researchers also would like to access, for the purpose of conducting the study, additional medical records concerning hospitalization or treatment, including, but not limited to, information regarding treatment for alcohol/substance abuse, human immunodeficiency virus (HIV), or for psychiatric treatment of counseling. *I understand that I have the right to specifically request that the above records NOT be released from my health care providers to the research team. I understand that, if I limit access to any of the records listed above, I may not be able to be in this research study.*

I do not authorize release of the following records to the research team:

____Mental Health Records _____Sexually Transmitted Disease
____Psychotherapy Notes _____Alcohol/Substance Abuse
____HIV (AIDS) _____ Other:_____

I understand that:

1. this authorization is valid for the duration of the study, including the analysis and publication or distribution of study results, unless I cancel the authorization as provided below.
2. efforts will be made to ensure that my protected health information will not be shared with other people outside the research study personnel indicated above. However, I recognize that my protected health information may be disclosed to others as required by law and/or to individuals or organizations that oversee the conduct of research studies, and these individuals may not be held to the same legal privacy standards as are doctors and hospitals. I understand, therefore, that the researchers cannot guarantee absolute confidentiality and privacy of my protected health information.

I understand that I have the right:

1. To refuse to sign this form. Not signing this form will not affect my regular health care including treatment, payment, or enrollment in a health plan or eligibility for health care benefits. However, not signing the form will prevent me from participating in the research study above.
2. To cancel this release of authorization at any time. If I choose to cancel this authorization, I must notify *Dr. Janet P. Wallace and Ryan Harris, MS at the Adult Fitness Program, HPER 070, Indiana University, Bloomington IN 47405; fax: 812 855-9417* in writing that I request a cancellation of this authorization. Even if I cancel this release of information authorization, the Research Team may still use information about me that was collected as part of the research project between the date I signed the current form, and the date I cancel the authorization. This is to protect the quality of the research results.
3. To receive a copy of this form.

I have had the opportunity to review and ask questions regarding this authorization form. By signing this authorization form, I am confirming that it reflects my wishes.

_____
(Printed name of Individual/Legal Representative)

_____
(Signature of Individual/Legal Representative) (Date)

**If signed by a legal representative: state the relationship and identify below the authority to act on behalf of the individual's behalf.*

Individual is a : ___Minor ____Incompetent ___Disabled ____Deceased
Legal Authority: ___Custodial Parent ___Legal Guardian ___Executor of Estate of Deceased ___Power of Health care Attorney ___Authorization of Legal Representative ___Other:_____

Please return the form to:

Ryan Harris, MS or Janet P Wallace, PhD
Clinical Exercise Physiology Laboratory- HPER 070
Indiana University
Bloomington, IN 47405-4801
Phone (812) 855-7556
Fax (812) 855-8179

RISK STRATIFICATION FORM

Name_____ Date _____ DOB_____ Age_____

Home Address_____ Phone_____

Email Address_____Phone (WORK / CELL) _____

Emergency Contact: Name_____ Relationship_____ Phone_____

Have you ever been told you have had a heart attack? _____Yes _____No
If yes, explain_____
Have you ever been told you have lung disease? _____Yes _____No
If yes, explain_____
Have you ever been told you have diabetes? _____Yes _____No
If yes, explain_____

MEDICATIONS
Please list all medications currently taking:

Medication	Reason	Dose

FAMILY HISTORY OF HEART DISEASE/STROKE/DIABETES
Indicate immediate family members (parents, siblings, aunt, uncles) who have diagnosed coronary heart disease/stroke and/or who have died from heart disease/stroke.
Check all that apply. Indicate age at diagnosis as appropriate.

	Heart Attack	Stroke	Diabetes
Father	☐ ____	☐ ____	☐ ____
Mother	☐ ____	☐ ____	☐ ____
Brother	☐ ____	☐ ____	☐ ____
Sister	☐ ____	☐ ____	☐ ____
Daughter	☐ ____	☐ ____	☐ ____

Do you smoke? _____Yes _____No

(Over)

Do you exercise? _____Yes _____No

Type of Exercise _____

How Often? _____days/week

How Long? _____min/session

How Hard? ☐ Low ☐ Moderate ☐ Hard

For Clinical Use Only:

WEIGHT

Height_____(in) Weight _____(lbs) Calculated BMI _____

BLOOD PRESSURE

Screening Blood Pressures _____/_____ mm Hg (average)

Day 1 Date_____	*Day 2* Date_____
_____/_____ mm Hg	_____/_____ mm Hg
_____/_____ mm Hg	_____/_____ mm Hg
_____/_____ mm Hg	_____/_____ mm Hg

CHOLESTEROL Date_____

Total Cholesterol	Current_____ mg/dL
LDL Cholesterol	Current_____ mg/dL
HDL Cholesterol	Current_____ mg/dL
Triglycerides	Current_____ mg/dL

BLOOD GLUCOSE Date_____ Glucose Current_____ mg/dL

For Office use only:

Risk Stratification: ☐ Unknown ☐ Low ☐ Moderate ☐ High

Physical Required: ☐ Vigorous Exercise ☐ Moderate Exercise ☐ Vigorous Exercise

Physician Supervision: ☐ Maximal Testing ☐ Maximal Testing ☐ Submax Testing

Evaluator: _____ Date:_____

MAXIMAL EXERCISE TEST DATA FORM

I.D.#_____Name_____ Date_____ Age_____ Sex_____

DOB _____ Physician_____ Do you smoke: Yes_____ No_____

Weight _____ lb. _____ kg. Height ___in. ____ cm. Fasting: Yes_____ No_____

Rest BP (mm Hg) #1_____/_____-_____ #2 _____/_____-_____ #3_____/_____

Recent illness, Chest Discomfort, or Arrhythmias, etc.

_____

Current Medications

_____

_____

Rest EKG_____ Axis_____* P-R _____ sec.

MD comments/Conclusions_____

Previous test :

Date	Speed	Final grade	Time	Peak HR	Peak VO_2	EKG

Time (min)	Grade (%) Load (rpm)	Rate (mph) (kpm/min)	Heart Rate (beats/min)	Blood Pressure mmHg Phase: 1/4-5	RPE	Ectopy	Comments
baseline				/ -			
0-2				/ -			
2-4				/ -			
4-6				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
peak				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			
				/ -			

Reason for termination_____

EXERCISE VARIABLES DATA COLLECTION FORM

Subject # _____ Date _____

VO2peak _____ ml/kg/min

Target VO2	25%	50%	75%
(ml/kg/min)			
± 5%			

Resting BP _____ / _____

Time (min)	HR (bpm)	BP (SBP/DBP)	RPE (6-20)	VO2 (ml/kg/min)
5		/		
10		/		
15		/		
20		/		
25		/		
30		/		
35		/		
40		/		
45		/		

Completed Exercise _____ minutes

Exercise Technician _____

FMD AND BIOMARKER DATA RECORD FORM

Subject # _____ Date _____

Visit # **1** **2** **3** _____

Time		***Baseline*** Diameter (mm)	Velocity (cm/s)	***Hyperemia*** Diameter (mm)	Velocity (cm/s)	**FMD (%)**	**Biomarkers** IL-6 (pg/ml)	TNF-a (pg/ml)
	Baseline					#DIV/0!		
	Immediately					#DIV/0!		
	1 hr post					#DIV/0!		
	2 hr post					#DIV/0!		
	3 hr post					#DIV/0!		

Sonographer _____

Measured By _____

APPENDIX H – DISSERTATION PROPOSAL

INTRODUCTION

The endothelium is a thin monolayer of cells that lines the walls of all the vessels in the body. Endothelial cells participate in multiple essential functions, including regulation of the vascular smooth muscle tone, control of thrombosis, inhibition of leukocyte and platelet adhesion, and promotion of intra-arterial permeability (4, 27, 36). A dysfunctional endothelium allows for adverse affects to the body's vasculature and has been associated with an increase risk for cardiovascular disease in many different clinical populations (9, 14, 19).

Within the past decade, the effects of exercise on endothelial function have received vast amounts of attention. Flow-Mediated Dilation (FMD) is a non-invasive measurement of endothelial function, which reflects the overall health and function of the arteries. There is substantial evidence suggesting that chronic exercise enhances endothelial function in healthy and clinical populations (12, 16); however, there is limited research investigating the effects of acute exercise on endothelial function. Harvey and colleagues (15) found that a single bout of exercise, at 60% VO_{2max} for 45 minutes, increases endothelial function approximately one hour post exercise with no additional endothelial function measurements taken.

The physiological mechanisms associated with the improvement in endothelial function are not fully understood (3). Furthermore, the FMD response beyond one hour of exercise cessation has not been investigated. Providing additional information past one hour post exercise may also provide valuable information to the possible mechanism influencing FMD following acute exercise.

The dose response of exercise can be divided into four different elements: mode, intensity, frequency, and duration. Each element may contribute in different ways in identifying possible exercise induced mechanisms associated with the improvement of endothelial function. For example, a strong association exists between training intensity, degree of oxidative stress, and inflammation (11, 17), all of which can impact FMD adversely. There has only been one study to investigate FMD following three different intensities (25%, 50%, and 75% VO_{2max}) of chronic exercise in humans (13). The findings of this investigation suggest that chronic cycling at moderate intensity (50% VO_{2max}) improves endothelial function, whereas cycling at low intensity (25% VO_{2max}) and high intensity (75% VO_{2max}) elicits no effect. Goto and colleagues (13) suggest that the increase in oxidative stress following high intensity exercise training impacted the FMD result, whereas moderate intensity exercise generated no change in oxidative stress. Furthermore, the 25% VO_{2max} may not provide a sufficient stimulus to influence oxidative stress or subsequent FMD. It is well known that the increase in oxygen uptake during acute exercise produces an increase in oxidative stress (OS) (37), which may appear to contradict the increase in FMD following exercise. In addition, evidence supports a strong correlation between oxidative stress and inflammation (23).

Inflammation has been found to increase cell surface adhesion and induce endothelial dysfunction (33). TNF-α is a pro-inflammatory cytokine that is elevated in patients with attenuated endothelial function (5, 18) and has been associated with the inflammatory pathogenesis of cardiovascular disease (33). There is evidence to support TNF-α's direct affect in producing endothelial dysfunction. Bhagat and colleagues (1) infused TNF-α into the arteries of healthy subjects and observed a transient decrease in microvascular

endothelial function. In addition, chronic endurance exercise has been found to attenuate TNF-alpha concentrations and reduce the acute inflammatory response (8). TNF-alpha is postulated to be the first responder (8) of the cytokine family to mediate OS induced by chronic exercise (2); however, the response of plasma TNF-alpha following acute exercise and its influence on FMD has not been investigated. Furthermore, IL-6 another proinflammatory cytokine, is typically the last inflammatory cytokine to respond (8). Until recently, IL-6 has been classified as both a pro- and anti-inflammatory cytokine; however, the current view accepts the primary role as having anti-inflammatory effects (26). Elevations in IL-6 concentrations may be due to increases in immune activity (29), unrelated inflammation (6, 29), catecholamine production (30), and exercise (20). Interleukin-6 acts to stimulate the hypothalamus-pituitary axis in response to inflammation, promotes osteoclastogenesis, and influences intermediary metabolism (20). The exercise-induced increase in IL-6 concentrations had been suggested to be a consequence of an immune response do to local damage in the working muscle (22), until recently debated.

Starkie and colleagues (31) have demonstrated that the immune cells are not the source of the increase in plasma IL-6 during exercise. Furthermore, Febbraio and colleagues (10) suggest the production of IL-6 in response to exercise is not an exercise induced immune response, it's a direct release from the muscle contraction itself. They refer to IL-6 as a myokine, a cytokine released from the skeletal muscle itself. The investigations from Pedersen and colleagues (25) support this phenomenon by suggesting that IL-6 is linked more so with metabolism (exercise) than it is with inflammation.

Furthermore, the production of IL-6 during exercise is related to the intensity and duration of the exercise (26).

Ullum and colleagues (35) report an increase in IL-6 in response to one-hour of cycle ergometry at 75% of maximal oxygen intake. In addition a 3 hour combination of cycle ergometry and treadmill running at 60-65% VO_{2max} increased IL-6 concentration greater than 18 fold compared to baseline values (21). Increases in IL-6 in response to cycling, treadmill, rowing, and eccentric exercise have all been reported (28). In addition, there appears to be a dose response relationship of amount of plasma IL-6 observed and exercise intensity (40% - 90%) with the greatest increase occurring at a relative intensity of ~75% (28).

There is conclusive evidence that IL-6 is released in response to all types, modes, and durations of exercise; however, the remaining unanswered question of why it is released is debated. During exercise there may be an increase in IL-6 from muscle to increase lipolysis and fat oxidation, but Pedersen and colleagues (25) report that IL-6 is not the sole mediator of these outcomes. IL-6 has also been shown to impair TNF-a expression in cardiac muscle (32) and skeletal muscle (26); consequently, the augmentation of endothelial function associated with acute and chronic exercise may be associated with the IL-6 mediated TNF-a suppression.

It is unclear if the elevated concentrations of IL-6 in response to exercise directly influence the endothelial response. The increase in concentrations of IL-6 in response to exercise may be a factor in controlling the pro-inflammatory response, thus limiting the TNF-α response, resulting in an improvement of endothelial function. Furthermore, the transient increase in oxidative stress observed following acute exercise does not explain

the increase in FMD. In addition, there appears to be no change in superoxide dismutase (34), total antioxidant status (37), or muscle antioxidant capacity (24) following acute exercise which would counteract the increase in OS and explain the augmentation of FMD. Nevertheless, IL-6 alone may not be the sole contributor that influences FMD following acute exercise. TNF-alpha may be the connecting link associated with the improvement in FMD. The mechanisms associated with the increase in endothelial function following acute exercise are unclear. The response of TNF-alpha following different acute exercise intensities may provide a link to the possible mechanisms associated with the FMD response following acute exercise.

PURPOSE

There are three purposes to this investigation; 1) to investigate the direct effect of the dose-response relationship of acute exercise on endothelial function, 2) to identify TNF-alpha and IL-6 as a possible link in the physiological mechanism in which endothelial function following acute exercise is augmented, and 3) to investigate the three hour time course of endothelial function and concentrations of TNF-alpha and IL-6 following acute exercise.

HYPOTHESES

Endothelial Function

It is hypothesized that:

- Exercise at 50% of VO_{2peak} will elicit the greatest endothelial response compared to 25% VO_{2peak} and 75% VO_{2peak}
- Exercise at 25% intensity will elicit no change in endothelial function
- Exercise at 75% intensity will attenuate endothelial function
- FMD will be augmented for the three hour time period studied following the 50% intensity treatment of acute exercise.

Biomarkers

It is hypothesized that:

- There will be an intensity associated increase in concentrations of IL-6 with acute exercise.
- There will be an intensity associated decrease in TNF-alpha with acute exercise.
- There will be an inverse relationship between concentrations of IL-6 and TNF-alpha that is dose dependent.
- Concentrations of IL-6 and TNF-alpha will be significant predictors of endothelial function.

DESIGN

In a randomized crossover design (figure 1), each subject will perform three different exercise interventions on three separate days within a two week period. For each intervention, Flow-Mediated Dilation (FMD) and subsequent blood samples will be taken at baseline, immediately following acute exercise, and every hour for three hours following the exercise intervention. FMD and blood samples will be used for the measurement and analysis of endothelial function and concentrations of IL-6 and TNF-alpha, respectively.

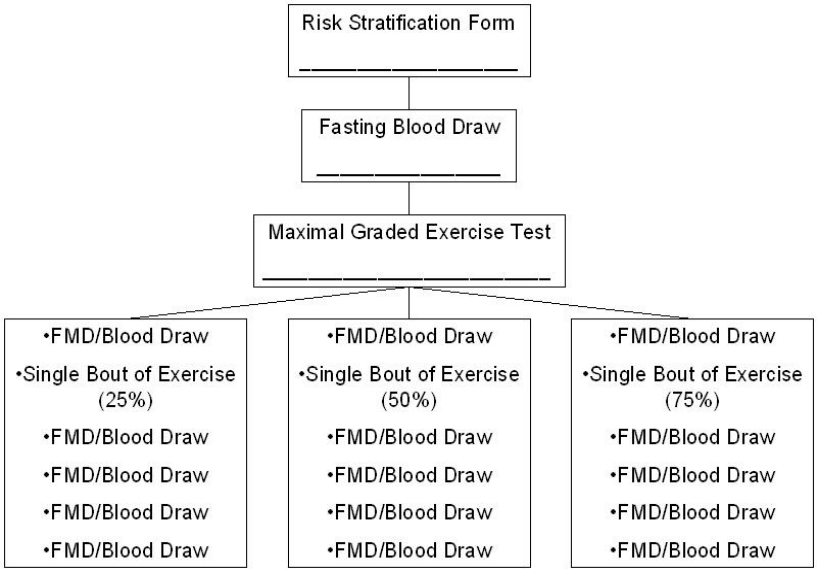


Figure 1. General Design

SUBJECTS

Approximately 15-20 moderate risk adults (18-60 yrs) will participate in the proposed investigation. Moderate risk, defined by the American College of Sports Medicine, are those men who are $\geq$ 45 years old or women $\geq$ 55 years old or any individuals who meet the threshold for two or more risk factors (ie: hypertension (BP $\geq$140/90),

hypercholesterolemia (TC >200mg/dl), smoking, sedentary lifestyle, family history of heart disease, obesity (BMI $\geq$30kg/m^2), and impaired fasting glucose (BG >100mg/dl)) for coronary heart disease.

Subject Number

The sample size is a conservative number to obtain significance in measuring endothelial function, TNF-alpha, and IL-6 based on published data (15, 20, 21) and pilot data from our laboratory.

Inclusion/Exclusion Criteria

Subject preparation, and FMD technique will follow the guidelines for ultrasound assessment of endothelial function (7). Inclusion into the study will require the subjects to:

- Be of moderate risk described by ACSM guidelines
- Have permission from their physician to participate in this investigation
- Not have any tobacco products at least 8hrs prior to each treatment day
- Not exercise for at least 12hrs prior to each treatment day
- Not have any caffeine at least 8hrs prior to each treatment day
- Not have any vitamin supplementation for at least 8hrs prior to each treatment day
- Not have any trace of vasoactive medications

Subjects will be excluded from the study if they:

- Are not between 18 and 60 years of age,
- Do not fall into the moderate risk classification (as described above),
- Cannot exercise at 75% VO_2peak for 45 minutes,
- Have a known allergy to heparin,
- Do not have peripheral venous access in the left upper extremity
- Have cardiovascular, pulmonary or metabolic diseases
- Have orthopedic problems that limit their exercise
- Take medications that dilate their arteries such as nitroglycerin, prostacyclin, or verapamil

PROCEDURES

Procedures to be used in this investigation are 1) risk stratification, 2) maximal exercise test, 3) acute exercise treatments, 4) measure of endothelial function, and 5) serial blood draws.

METHODS

Risk Stratification

The **risk stratification form** summarizes information about the subject's medical history, present diseases/disorders, family history of disease, height and weight, and current medications along with information that will be obtained from each subjects fasting blood draw.

A **fasting blood draw** performed at the Indiana University Health center will be required before any subject can participate in the study. This test is to help determine the risk stratification for exercise. Each subject will report to the Indiana University Health Center (Room 208) and must be fasting for at least 12 hours prior to this test. A 20-45 ml sample of venous blood will be drawn by a certified technician via sterile techniques for analysis of cholesterol, triglycerides and glucose.

Maximal Exercise Test

A **maximal graded exercise test** will be performed to obtain the appropriate exercise treatment intensities. This test is designed to measure the subjects maximal exercise capacity. Each subject will walk or jog on a motor-driven treadmill beginning at a speed between 2.5-5.0 mph, 0% grade, with the grade or slope increasing 2.5% every two minutes until volitional fatigue, breathlessness, chest discomfort, and/or any other symptoms which indicate to yourself or the technicians that you should stop exercise. Heart rate via 12-lead EKG will be monitored throughout the test. Blood pressure will be

taken before, during if walking (every stage), and after the test (recovery). Expired gases will also be collected through a unidirectional flow mouthpiece during the test.

Acute Exercise Treatments

Subjects will be asked to perform three 45-minute **walking exercise session**s at 25%, 50%, and 75% of their exercise capacity (on three separate days) obtained from the maximal graded exercise test. Expired gases will be measured through a unidirectional flow mouthpiece during the 5th minute of each exercise session to confirm their exercise intensity. The work rate will be adjusted if it is not within ±10% of your target exercise intensity. Expired gases will then be measured again during the 15th minute to confirm the new exercise intensity. Heart rate and blood pressure will be monitored throughout each exercise session.

Endothelial Function

For each acute exercise treatment, brachial artery **flow-mediated dilation** will be used as a measurement of endothelial function. Flow-mediated dilation will be measured at baseline, immediately post acute exercise and 1, 2, and 3 hours post acute exercise. Each subject will lie on their back for 20 minutes to establish a hemodynamic resting state. After the 20 minute acclimation phase, a water based silicon gel will be placed on the ultrasound transducer as an impedance adapter for better ultrasound images. The Sonoace Pico ultra sound system using a 7.0 MHz linear transducer will then be used to scan the baseline artery of their inner arm, 2-10 cm above the elbow. Once a clear image is obtained, resting blood flow velocity and a baseline image of their artery will be captured by the ultrasound. After baseline measurements are captured, a second blood pressure cuff will be wrapped around the forearm of the right arm and inflated to 250 mm Hg to stop blood flow to the lower arm for 5 minutes. Blood flow velocity will be

recorded 10 seconds after the cuff is deflated and ultrasound images will be captured as described above. The health of the artery will be expressed as a percentage of the artery expansion with the increased blood flow, compared to the resting diameter. **The dependent variables will be brachial artery diameter(mm), absolute change in brachial artery diameter(mm), and FMD (%)**.

Serial Blood Draws

For the **serial blood draw**, a 22-24 gauge venous catheter will be inserted in a vein in the subjects left arm by a clinical exercise physiologist who has special IV training and practice. The catheter will remain there for the entire duration of the testing day (approximately 6-8 hrs). The catheter will be flushed with normal saline, and a small blood sample (15-20ml) will be collected from the inserted catheter and stored for the analysis of IL-6 and TNF-alpha. For each measurement of FMD performed a subsequent blood sample will also be collected. Following each blood draw, the IV will be flushed with a heparin based solution to prevent clotting of the catheter. Once the testing day is completed, the catheter will be removed by the same trained clinical exercise physiologist.

Analysis of IL-6 and TNF-alpha

The analysis of IL-6 and TNF-alpha will be performed using a high sensitivity Enzyme Linked Immunoassay (ELISA) kit and ran in triplicate according to manufacture specifications (R & D Systems, Mineapolis, MN). Briefly, this assay utilizes the quantitative sandwich technique. An antibody is coated to the plate and any plasma IL-6 or TNF-alpha introduced into the plate is bound by the immobilized antibody. Following a wash of any unbound antibody-enzyme reagent, a substrate is introduced into the well with subsequent amplification for color development in proportion to the amount of IL-6

or TNF-alpha is bound. The color absorbance is plotted against the standard curve to obtain concentrations of IL-6 and TNF-alpha. **The dependent variables will be plasma concentrations of IL-6 (pg/ml) and TNF-alpha (pg/ml).**

STATISTICAL METHODS

Demographics will be analyzed using descriptive statistics. All data will be expressed as mean ± SD. Significance will be set at $p < .05$.

Endothelial Function

To investigate the hypotheses evaluating the time course associated with 50% exercise intensity and the endothelial response associated with the dose of exercise, a two-way (intensity x time) repeated measures ANOVA will be performed.

Biomarkers

To investigate the relationship between IL-6, TNF-alpha and exercise intensity and the relationship between IL-6 and TNF-alpha, three Pearson Product Moment Correlations will be performed: 1) between IL-6 and intensity, 2) between TNF-alpha and intensity, and 3) between IL-6 and TNF alpha. All exercise intensities will be incorporated into each correlation. To investigate if concentrations of IL-6 and TNF-alpha are significant predictors of endothelial function, regression analysis will be performed.

SIGNIFICANCE

Endothelial dysfunction is seen in various clinical conditions such as hypertension, diabetes, coronary artery disease, and obesity. The role of exercise as a non-pharmacological intervention is becoming more important in the treatment and prevention of disease. The present study may 1) contribute in identifying a link to the possible mechanism associated with the improvement of FMD following acute exercise

and 2) indicate an appropriate exercise stimulus that will not exacerbate the inflammatory process, yet improve the endothelial response. As clinicians, the more comprehensive understanding we have about the dose response of exercise, and its association with endothelial function, the more effective we can prescribe exercise as a non-pharmacological intervention in combating and treating disease.

REFERENCES

1. Bhagat, K. and P. Vallance. Inflammatory Cytokines Impair Endothelium-Dependent Dilatation in Human Veins In Vivo. *Circulation*. 96:3042-3047, 1997.
2. Bilsborough, W., G. O'Driscoll, K. Stanton, R. Weerasooriya, L. Dembo, R. Taylor, and D. Green. Effect of lowering tumor necrosis factor-a on vascular function in type II diabetes. *Clin Sci*. 106:163-169, 2002.
3. Brunner, H., J. R. Cockcroft, J. Deanfield, A. Donald, E. Ferrannini, J. Halcox, W. Kiowski, T. F. Luscher, G. Mancia, A. Natali, J. J. Oliver, A. C. Pessina, D. Rizzoni, G. P. Rossi, A. Salvetti, L. E. Spieker, S. Taddei, and D. J. Webb. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the working group of endothelins and endothelial factors of the european society of hypertension. *J Hypertens*. 23:233-246, 2005.
4. Celermajer, D. Endothelial dysfunction: does it matter? is it reversible? *Journal of American College of Cardiology*. 30:325-333, 1997.
5. Conraads, V. M., P. Beckers, J. Bosmans, L. S. DeClerck, W. J. Stevens, C. J. Vrints, and D. L. Brutsaert. Combined endurance/resistance training reduces plasma TNF-alpha receptor levels in patients with chronic heart failure and cornary artery disease. *Eur Heart J*. 23:1854-1860, 2002.
6. Corpeleijn, E., W. H. M. Saris, E. H. J. M. Jansen, P. M. H. J. Roekaerts, E. J. M. Feskens, and E. E. Blaak. Postprandial Interleukin-6 Release from Skeletal Muscle in Men with Impaired Glucose Tolerance Can Be Reduced by Weight Loss. *J Clin Endocrinol Metab*. 90:5819-5824, 2005.

7. Corretti, M. C., T. J. Anderson, E. J. Benjamin, D. Celermajer, F. Charbonneau, M. A. Creager, J. Deanfield, H. Drexler, M. Gehard-Herman, D. Herrington, P. Vallance, J. Vita, and R. Vogel. Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery. *J Am Coll Cardiol*. 39:257-265, 2002.

8. Drenth, J. P., S. H. Van Uum, M. Van Deuren, G. J. Pesman, J. Van der Ven-Jongekrijg, and J. W. Van der Meer. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production. *J Appl Physiol*. 79:1497-1503, 1995.

9. Edwards, D. G., R. S. Schofield, S. L. Lennon, G. L. Pierce, W. W. Nichols, and R. W. Braith. Effect of exercise training on endothelial function in men with coronary artery disease. *Am J Cardiol*. 93:617-620, 2004.

10. Febbraio, M. A. and B. K. Pedersen. Contraction induced myokine production and release: Is skeletal muscle an endocrine organ. *Exerc Sport Sci Rev*. 33:114-119, 2005.

11. Forjaz, C. L., C. G. Cardoso, C. C. Rezk, D. F. Santaella, and T. Tinucci. Postexercise hypotension and hemodynamics: the role of exercise intensity. *J Sports Med and Phys Fit*. 44:54-62, 2004.

12. Gokce, N., J. A. Vita, D. S. Bader, D. L. Sherman, L. M. Hunter, M. Holbrook, C. O'Malley, J. F. Keany, and G. J. Balady. Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am J Cardiol*. 90:124-127, 2002.

13. Goto, C., Y. Higashi, M. Kimura, K. Noma, K. Hara, K. Nakagawa, M. Kawamura, K. Chayama, M. Yoshizumi, and I. Nara. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans. Role of endothelium-dependent nitric oxide and oxidative stress. *Circulation*. 108:530-535, 2003.

14. Guerci, B., A. Kearney-Schwartz, P. Bohme, F. Zannad, and P. Drouin. Endothelial dysfunction and type 2 diabetes. *Diabetes Metab*. 27, 2001.

15. Harvey, P. J., B. L. Morris, T. Kubo, P. E. Picton, W. S. Su, C. F. Notarius, and J. S. Floras. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. *Hypertension*. 23:285-292, 2005.

16. Kasikcioglu, E., H. Oflaz, H. A. Kasikcioglu, A. Kayserilioglu, S. Umman, and M. Meric. Endothelial flow mediated dilation and exercise capacity in highly trained endurance athletes. *Tohoku J of Exp Med*. 205:45-51, 2005.

17. Kemi, O. J., P. M. Haram, J. P. Loennechen, J. Osnes, T. Skomedal, U. Wisloff, and O. Ellingsen. Moderate vs high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovasc Res*. 67:161-172, 2005.

18. Lee, I. M., H. D. Sesso, Y. Oguma, and R. S. Paffenbarger. Relative intensity of physical activity and reisk of coronary heart disease. *Circulation*. 107:1110-1116, 2003.

19. Maiorana, A., F. O'Droscoll, R. Taylor, and D. Green. Exercise and the Nitric Oxide Vasodilator System. *Sports Med*. 33:1013-1035, 2003.

20. Minetto, M., A. Rainoldi, M. Gazzoni, M. Terzolo, P. Borrione, A. Termine, L. Saba, A. Dovio, A. Angeli, and P. Paccotti. Differential responses of serum and salivary interleukin-6 to acute strenuous exercise. *European Journal of Applied Physiology*. 93:679-686, 2005.

21. Moldoveanu, A., R. J. Shephard, and P. N. Shek. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol*. 89:1499-1504, 2000.

22. Nieman, D. C., S. L. Nehlsen-Cannarella, O. R. Fagoaga, D. A. Hanson, A. Utter, J. M. Davis, F. Williams, and D. E. Butterworth. Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc*. 30:671-678, 1998.

23. Niess, A. M., H. H. Dickhuth, H. Northoff, and E. Fehrenbach. Free radicals and oxidative stres in exercise-immunological aspects. *Exerc Immunol Rev*. 5:22-56, 1999.

24. Parise, G., S. M. Phillips, J. J. Kaczor, and Tarnopolsky. Antioxidant enzyme activity is up-regulated after unilateral resistance exercise training in older adults. *Free Rad Bio Med*. 39:289-295, 2005.

25. Pedersen, B. K., A. Steensberg, C. Fischer, C. Keller, P. Keller, P. Plomgaard, E. Wolsk-Peterson, and M. Febbraio. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Preceedings of the Nutrition Society*. 63:263-267, 2004.

26. Pedersen, B. K., A. Steensberg, and P. Schjerling. Muscle-derived interleukin-6: possible biological effects. *J Physiol*. 536:329-337, 2001.

27. Rubanyi, G. M. The role of endothelium in cardiovascular homeostasis and disease. *Journal of Cardiovascular Pharmacology*. 22:S1-S14, 1993.

28. Shephard, R. J. Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. *Crit Rev Immunol*. 22:165-182, 2002.

29. Shephard, R. J. Sepsis and mechanisms of inflammatory response: Is exercise a good model. *Br J Sports Med*. 35:223-230, 2001.

30. Starkie, R., S. R. Ostrowski, S. Jauffred, M. Febbraio, and B. K. Pedersen. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha; production in humans. *FASEB J.*:02-0670fje, 2003.

31. Starkie, R. L., J. Rolland, D. J. Angus, M. J. Anderson, and M. A. Febrraio. Circulating moncytes are not the source of elvations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Cell Physiol*. 280:C769-C774, 2001.

32. Steensberg, A., C. Keller, R. L. Starkie, T. Osada, M. A. Febbraio, and B. K. Pedersen. IL-6 and TNF-a expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab*. 283:E1272-E1278, 2002.

33. Stenvinkel, P. Endothelial dysfunction and inflammation-is there a link? *Nephrol Dial Transplant*. 16:1968-1971, 2001.

34. Tozzi-ciancarelli, M. G., M. Penco, and C. D. MAssimo. Influence of acute exercise on human platelet responsiveness: possible involvement of exercise-induced oxidative stress. *Eur J App Physiol*. 86:266-272, 2002.

35. Ullum, H., P. M. Haahr, M. Diamant, J. Palmo, J. Halkjaer-Kristensen, and B. K. Pedersen. Bicycle exercise enhances plasma IL-6 but does not change IL-1 alpha,

IL-1 beta, IL-6, or TNF-alpha pre-mRNA in BMNC. *J Appl Physiol*. 77:93-97, 1994.

36. Vane, J. R., E. E. Anggard, and R. M. Botting. Regulatory functions of the vascular enodothelium. *The New England Journal of Medicine*. 323:27-36, 1990.

37. Vincent, H. K., J. W. Morgan, and K. R. Vincent. Obesity exacerbates oxidative stress levels after acute exercise. *Med Sci Sports Exerc*. 36:772-779, 2004.

APPENDIX I – RESEARCH PROCEDURES – STEP BY STEP

BRACHIAL ARTERY FMD – STEP BY STEP

The gold standard of measuring flow mediated dilation (FMD) is with a high resolution ultrasound device that is found in the Clinical Exercise Physiology Laboratory. The purpose of this paper is to guide you step by step through the Indiana University CEP lab Flow Mediated Dilation protocol ***(updated 5/15/06 for use with MIA).***

INCLUSION CRITERIA

The measurement of flow mediated dilation requires a few steps of preparation. It is important that you review the subjects' medical/health history questionnaire. The subject should:

- Not engage in exercise for at least 12 hrs before study
- Ingest no caffeine at least 4-6hrs before study
- Ingest no Vitamin C at least 4-6hrs before study
- Not smoke any tobacco products at least 4-6hrs before study
- Be fasting for 8 hrs prior to the study
- Not be on any vaso reactive medications for the past 4 half-lives

Once you have determined that the subject meets inclusion criteria for the measurement of flow-mediated dilation proceed to the initial steps of collecting data.

SUBJECT PREPARATION

The first step in preparing the subject for data collection is to go over the informed consent form. Once the subject has agreed and consented to the procedure, you can fill out a data collection sheet with the following information:

- ID
- Age
- Height
- Weight
- BMI
- Cholesterol
- VO_{2Peak}
- Check all risk factors that apply

The next step is to have the subject lie supine on the table for a 20 minute acclimation phase (Feet towards the wall and head towards the door) with their right arm extended towards the ultrasound machine. While the subject is lying supine, place the Hokanson SC-5 rapid cuff around the subject's right forearm.

Open the Time Left program for desktop timer.

Entering Subject ID

You will need to open the imager program on the MIA. Once in imager you will need to set up the study:

- Action
  - Setup Study (F2) – Fill out all necessary fields including the file name. You will need to discuss how you will code the study with your research team. Once all necessary fields are filled in, click save. *Note: you will need two file names for each FMD performed; one for baseline and one for post occlusion.*

Information to enter in the saved file:

- Study ID (Abbreviation of the study)
- Condition (BA baseline or BA deflation)
- Reader ID (Name of Technician)
- Subject ID (Research #)
- Image File (Study ID_Research #_Visits (1, 2, 3…(treatments will be randomized)_Time (A, B, C…)_FMD Condition(1=baseline **or** 2=deflation)
- **Example: BP_R2050_2_C_2**
  - **All ID's will have 5 series of #'s. If the study only has one visit, use 1 as a place holder, etc.**

Now you are ready to start acquiring images. Make sure you are set in Trigger Mode and set up the frame grabber as follows:

Baseline: 30 Frames

Post Occlusion: 200 Seconds

DATA COLLECTION

Baseline Data

For baseline images you should set up your grabber to automatically grab 30 frames (10 frames for Doppler and 10 frames for 2D). It will be important to capture your Doppler pulse waves first. The baseline diameter is used as the "Pre" measurement when calculating the flow mediated dilation. Place the linear transducer longitudinally along the brachial artery 2-10cm above the antecubital fossa. In "**C"** mode obtain and verify the artery by visually seeing the color flow. After verification of the brachial artery switch to "**PW"** mode and press the camera icon or F1 to begin capturing 10 triggered frames. When finished capturing 10 frames press "**2D**" and continue capturing 10 triggered frames of the BA.

Note: To obtain baseline BA values, average all acceptable diameter frames to represent the baseline diameter.

Flow Mediated Dilation

When you're finished collecting baseline data you will need to set the rapid cuff inflator to 250 mm Hg and press to inflate when ready. Maintain the occlusion for 5 minutes while maintaining the brachial artery image on the ultrasound screen. *Note:*

During this 5 minute occlusion period you should set up your post occlusion file in the imager program. For post occlusion images you should set up your grabber to grab for a period of time; 200 seconds with the first 10 triggered frames being for Doppler and the rest of the 2 minutes post occlusion in 2D mode

After the first 4.5 minutes of occlusion start the image grabbing (camera or F1). After 5 minutes of occlusion, press to rapidly deflate the forearm cuff while maintaining the image on the screen (It is important not to lose the image as the rapid velocity of the blood will distort the placement of the artery relative to the ultrasound transducer). Maintain the image on the screen for a total of 2 minutes post occlusion and then press F1 or the little camera icon to stop frame grabbing.

DATA ANALYSIS (after completion of the study)

There may be two observers involved with the data analysis to ensure inter-reliability of the measurements taken.

You will need to open the file you want to measure in the Brachial Tools program:

- Open brachial tools
    - File → open → locate file you want to analyze

Once the file is open in brachial tools you will need to

- Train the BA
- Measure the BA
- Train the Doppler
- Measure the Doppler

Note: First calibrate, define the ROI, and analyze BA (as described below) before you analyze the Doppler flow.

Train and measure the Brachial Artery

The next step will be to calibrate the brachial artery.

- Action → Calibrate
    - Calibrate vertical axis
- Action → Initialize Define ROI
    - Brachial artery ROI- Position the ROI so that the middle of the circle is in the center of the artery. *Note: It is important to make sure the ROI will be ok to analyze the post deflation artery if needed.*

Once you have defined the ROI,

- Click next → Proceed → Cancel

Click the Launch automated analysis (shuttle icon)and click the play button. This will start the analysis where you started and continue through the end of the captured frames. You can start and stop this manual analysis as you wish.

Brachial artery Values

After analyzing the BA you will have a diameter value for each frame grabbed. The Confidence Interval (CI) of the computer must be $\geq$ 70%. You can click to eliminate all CI that are below 70%. You can also eliminate a value manually. If the eliminated

values represent greater than 80%, you need to rerun your analysis. *Note: You can go frame by frame and manually measure the frame that fell out of the 70% CI the same way you trained the ROI.*

Note: To obtain the post hyperemia value, look at the BA diameters from 45 seconds post deflation (first hyperemic flow) to the end of the captured frames. Follow these steps

- *Take the highest average of the 10 second interval to be used as this value.*
- *You can obtain the 10 second intervals when you create an Excel report through the subject manager.*

Train and measure the Doppler

With the Doppler image on the screen click

- Action → Start flow analysis
  - Calibrate Velocity (vertical) → Finish
  - Calibrate Time (horizontal) → Finish
  - Define Flow ROI

Once you have calibrated and defined the ROI for the flow envelope click Analyze. This will start to measure the envelope that you have trained for the Doppler flows. Stop the analysis when no more Doppler frames are present.

To edit, select each frame analyzed and reject the frames that have either a duplicated tracing or do not trace appropriately. Click save to save all measurements analyzed.

Exporting to Excel

After all analysis for the visit day are completed, you can export all files into one excel spreadsheet for easier data manipulation. Click on

- Subject Information → new → add all files → Save report as follows:
  - Research #_visit #_Final
  - Example: R2059_2_Final

Results

Results are presented as the percent change from baseline. Alternate results may include:

- Baseline diameter
- Absolute change in diameter
- Percent change in diameter

Intra and Inter observer reliability should be established by the Intra Correlation Coefficient (ICC)

VENIPUNCTURE AND BLOOD CENTRIFUGATION – STEP BY STEP

Supplies Needed:

- Alcohol
- Tape
- Gauze
- Angiocath
- PRN adapter
- Band aids
- Gloves
- Tourniquet
- Normal Saline (NS)
- Heparin Flush (HEP)
- Syringe
- Needle
- Sharps Container
- Blood Collection Tubes

Preparation

It is important that all of your supplies are prepared and ready to use. For each venipuncture attempt you will need: (1) angiocath, (1) PRN adapter, (1) NS syringe and needle (~5ml), (1) HEP syringe and needle (~5ml), (1) band aid, (3) pieces of tape (2 thin, 1 thick), (2) empty syringes, (1) EDTA blood collection tube, (2-3) alcohol pads, a tourniquet, a pair of gloves and some gauze.

Drawing up Fluid

Wipe the top of the vile with alcohol and allow it to dry. Assemble the needle and syringe without touching the site that the two connect. Pull back the syringe according to how much fluid you want to draw up. Insert the needle into the vile and plunge the air from the syringe into the vile. This will create a vacuum and fluid will start to transfer from the vile into the syringe. Draw back on the syringe to the exact amount you desire. Pull the needle out of the vile and remove all the air bubble remaining in the fluid by taping or "flicking" the syringe. Plunge the syringe until the fluid is released at the tip of the needle and recap. This syringe is ready to use.

Venipuncture

First you want to explain to the subject the procedure and what your about to do to him/her. With your gloves off make sure that your angiocath, PRN adapter, band aid, and tape are all prepared ready to use. Tie the tourniquet on the arm superior to the site you are targeting and allow a few seconds for the vein to expand up towards the surface. Palpate the vein and asses whether or not you want to proceed. If not, start over and place the tourniquet superior to the site you want to attempt. If you feel that the site is appropriate than alcohol the immediate and surrounding area of the vein in a dabbing, yet rough manner attempting to clean the area thoroughly.

Put your gloves on (allowing for the alcohol to dry) and remove the cap from the angiocath. Tell the subject, "your going to feel a little stick," and stick the subject at a very obtuse angle in a smooth, constant motion. Watch for blood return in the angiocath window. Once you see a return, advance the catheter 1-2 more millimeters, and thread the catheter all the way to the base of the skin. Remove the tourniquet. As you remove the needle from the site, apply pressure directly over the site where you inserted the

catheter (this will slow the blood entering the open angiocath). Discard the needle into the sharps container and quickly replace the needle with a PRN adapter.

Using one of the thin pieces of tape, place it over the PRN adapter securing it in place. Put your NS syringe into the PRN adapter and slowly flush 1-2 ml of fluid. Draw back and you should see some blood enter the syringe. Once this happens, you know you have accessed the vein, and flush the remaining volume from the syringe into the vein. Place the band aid directly over the base of the catheter, close to the skin, and using the other 2 pieces of tape, tape the catheter securely in place.

Drawing up your Sample

When you're ready to draw up your blood sample, alcohol the PRN adapter (you don't have to alcohol the adapter if you have just entered the vein and are drawing a sample. Only if you have the subject is returning after some period of time), put the tourniquet on, place a piece of gauze under the PRN adapter, and put the needle of one of your empty syringes into the PRN adapter. Slowly draw up 10 ml of blood, and discard the entire syringe. Put the needle of your other empty syringe into the PRN adapter and slowly draw up 10 ml of blood. Stick the needle into the vaccutainer blood collection tube and the blood will passively fill the tube. Invert the tube 4-5 times slowly; mixing the EDTA with the blood so it will not clot. Discard the empty syringe and needle into the sharps container.

Centrifuging and Storage of Blood

Within 60 minutes of collection into the EDTA tube, plasma needs to be separated. Place the sample tube into the Beckman centrifuge (HPER 076) with an appropriate balance (it can be another sample or a water balance). Centrifuge each sample at a rate of 1300 RPM for 12 minutes. Once you have separation of plasma and RBC's, pipette 1 ml each into 3 separate microfuge tubes. Close caps, and label each tube correctly. Store all plasma samples in -80° C until analysis.

Removing the Angiocath

When you're ready to remove the catheter, try to remove most of the tape and band aid. Put an alcohol pad over a piece of gauze and place them over the site of entry with the alcohol towards the skin. As you slowly remove the catheter, apply firm pressure to the site. Hold the pressure for 30-120 seconds and then cover with a band aid for at least 30 minutes.

R AND D IL-6 ASSAY PROCEDURE – STEP BY STEP

It is very important to wear a dust mask and change gloves throughout the assay frequently!!!

Before you begin, bring all reagents and samples to room temperature before use. If setting up two or more separate plates, Set up all reagents and standards first, and then start one plate set up… (incubate 2 hours), minutes and start the second or third one immediately.

Items you will need per kit:

- P100, P1000, L300
- Vortex
- Pipette aid
- Dust masks
- 7 microfuge tubes
- 1 boxes of 100µl tips
- 2.5 boxes of 300µl multi-channel tips
- 2 syringes/needles
- 1000ml volumetric flask
- 1000ml-1500ml tip waste beaker
- 1 5ml pipettes
- 1 10ml pipettes
- 5 Reagent Reservoirs

REAGENT PREPARATION

1) **Calibrator Diluent RD6-11** – Dilute 10ml of calibrator diliuent concentrate into 10ml of ultra pure water using a **10ml pipette** (total 20ml) and a **40ml beaker.**

2) **IL-6 Standard** – Reconstitute the IL-6 standard with 5ml of **calibrator diluent** using a **5ml pipette.** *This reconstitution will be your stock solution of 10pg/ml.* **Allow the stock solution to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.**

3) **Wash Buffer –** Dilute 100ml of **wash buffer concentrate** into 900ml of ultra pure water to prepare 1000ml of wash buffer using a **1000ml volumetric flask.**

4) **Substrate Solution** – Using a **10ml syringe and needle** reconstitute 6ml of **substrate diluen**t into the **lyophilized substrate** at least 10 minutes before use. ***Discard Stopper after reconstitution.***

5) **Amplifier Solution** - Using a **10ml syringe and needle** reconstitute 6ml of **amplifier diluen**t into the **lyophilized amplifier** at least 10 minutes before use. ***Discard Stopper after reconstitution.***

6) **Creating the Standards** – If 15 minutes has passed, **using the same pipette tip**:

    a. Pipette 500µl **(P1000)** of **calibrator diluent** into (7) sterile **microfuge tubes**.

b. Use the stock standard solution and serially dilute 500µl of 10pg/ml into the tube labeled 5pg/ml and mix thoroughly.
c. Take 500µl out of the 5pg/ml and transfer into the 2.5pg/ml tube and mix thoroughly.
d. Continue all they way down to 1.25pg/ml, 0.625pg/ml, 0.312pg/ml, and 0.156pg/ml.
e. Do not put anything into the 0 tube which should contain only 500µl of calibrator diluent.

ASSAY PROCEDURE

It is recommended that all samples be assayed in duplicate

Once all reagents and standards are prepared as instructed:

1) Using the **multi-channel pipette** and a **reservoir**, add 100µl of **assay diluent** to each well. **Change tips after every row.** *Be sure to mix the assay diluent prior to emptying into the reservoir.*

2) Using the **P100**, add 100µl of standard or sample to each well. Cover with adhesive strip and incubate for **2 hours** at room temperature on an orbital shaker set at 500rpm. *Gently mix standards and samples prior to adding into the well.*
3) Wash 6 times (see wash procedure below)

4) Using the **multi-channel pipette** and a **reservoir**, add 200µl of **conjugate** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **2 hours** at room temperature on an orbital shaker set at 500rpm. *Be sure to mix the conjugate prior to emptying into the reservoir.*

5) Wash 6 times

6) Using the **multi-channel pipette** and a **reservoir**, add 50µl of **substrate solution** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **60 minutes** at room temperature **on the benchtop**. *Be sure to mix the substrate solution prior to emptying into the reservoir.*

7) Using the **multi-channel pipette** and a **reservoir**, add 50µl of **amplifier solution** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **30 minutes** at room temperature **on the bench top**. *Be sure to mix the amplifier solution prior to emptying into the reservoir.*

8) Using the **multi-channel pipette** and a **reservoir**, add 50µl of **stop solution** to each well. **Change tips after every row.** *Read at 490nm and 690nm within 30 minutes.*

WASH PROCEDURE

Inclusion of a 30 second soak between each addition of wash buffer and decanting will improve the precision of the assay

1) Remove liquid from the wells by inverting the plate and decanting the contents
2) Remove excess liquid by grasping the plate firmly and **smartly** rapping the plate inverted on a clean paper towel at least 5 times
3) Fill each well with 400µl of **Wash Buffer** (Use Plate Washer setting R-D)
4) Repeat for a total of 6 washes.
5) After the last wash, **smartly** rap the inverted plate on a clean paper towel at least 10 times to remove excess **Wash Buffer**.

PLATE READER

Running the Program

Protocol

- Reading Parameters
  - Endpoint
  - Read mode: normal
  - Need to wavelengths, 490nm and 690nm
- Plate Layout
  - Standard: put units and values of concentrations from kit book
- Curve
  - Axis: extrapolation factor **3.0**
  - Curve Fit: Linear or whatever the book says
  - **This kit does not use blanks. Use multi plate transformation to subtract Std 0 from the samples. Also use Curve interpretation to interpret Delta OD. Use Protocol R and D IL-6 (refer back to the IL-6 manual and the plate reader manual for instructions)**

Reading

- System: Administrator login: Admin
- Display: curves, allows you to look at your standard curve

Save, export

R AND D TNF-α ASSAY PROCEDURE – STEP BY STEP

Before you begin, **bring all reagents and samples to room temperature before use**. If setting up two separate plates, Start one plate set up… (incubate 3 hours), wait 15 minutes and start preparing the reagents and set up plate #2.

Items you will need per kit:

- P200, P1000, L300
- Vortex
- 7 microfuge tubes
- 1 boxes of 200µl tips
- 2.5 boxes of 300µl multi-channel tips
- 2 syringes/needles
- 1000ml volumetric flask
- 1000ml-1500ml tip waste beaker
- ~2 Disposable Pasteur pipettes
- 1 5 ml pipette
- 5 reagent reservoirs

REAGENT PREPARATION

7) **TNF-alpha Standard** – Reconstitute the TNF-alpha standard with **the volume of calibrator diluent printed on the standard vial label** using a **5 ml pipette.** *This reconstitution will be your stock solution of 32pg/ml.* **Allow the stock solution to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.**

8) **Wash Buffer –** Dilute 100ml of **wash buffer concentrate** into 900ml of ultra pure water to prepare 1000ml of wash buffer using a **1000ml volumetric flask.**

9) **Substrate Solution** – Using a **10ml syringe and needle** reconstitute 6ml of **substrate diluen**t into the **lyophilized substrate** at least 10 minutes before use. ***Discard Stopper after reconstitution.***

10) **Amplifier Solution** - Using a **10ml syringe and needle** reconstitute 6ml of **amplifier diluen**t into the **lyophilized amplifier** at least 10 minutes before use. ***Discard Stopper after reconstitution.***

11) **Creating the Standards** – If 15 minutes has passed, **using the same pipette tip**:

    a. Pipette 500µl of **calibrator diluent** into (7) sterile **microfuge tubes**.
    b. Use the stock standard solution and serially dilute 500µl of 32pg/ml into the tube labeled 16pg/ml and mix thoroughly.
    c. Take 500µl out of the 16pg/ml and transfer into the 8pg/ml tube and mix thoroughly.
    d. Continue all they way down to 4pg/ml, 2pg/ml, 1pg/ml, and 0.5pg/ml.
    e. Do not put anything into the 0 tube which should contain only 500µl of calibrator diluent.

ASSAY PROCEDURE

It is recommended that all samples be assayed in duplicate and you wear a mask and gloves throughout the procedure.

Once all reagents and standards are prepared as instructed:

9) Using the **multi-channel pipette** and a **reservoir**, add **5**0µl of **assay diluent** to each well. **Change tips after every row.** *Be sure to mix the assay diluent prior to emptying into the reservoir.*

10) Using the **P200**, add **200µl** of standard or sample to each well. Cover with adhesive strip and incubate for **3 hours** at room temperature. *Gently mix standards and samples prior to adding into the well.*

11) Wash 6 times (see wash procedure below)

12) Using the **multi-channel pipette** and a **reservoir**, add **200µl** of **TNF-a HS conjugate** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **2 hours** at room temperature. *Be sure to mix the conjugate prior to emptying into the reservoir.*

13) Wash 6 times

14) Using the **multi-channel pipette** and a **reservoir**, add **50µl** of **substrate solution** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **60 minutes** at room temperature. *Be sure to mix the substrate solution prior to emptying into the reservoir.*

15) Using the **multi-channel pipette** and a **reservoir**, add **50µl** of **amplifier solution** to each well. **Change tips after every row.** Cover with adhesive strip and incubate for **30 minutes** at room temperature **on the bench top**. *Be sure to mix the amplifier solution prior to emptying into the reservoir.*

16) Using the **multi-channel pipette** and a **reservoir**, add **50µl** of **stop solution** to each well. **Change tips after every row.** *Read at 490nm and 690nm within 30 minutes.*

WASH PROCEDURE

Inclusion of a 30 second soak between each addition of wash buffer and decanting will improve the precision of the assay

6) Remove liquid from the wells by inverting the plate and decanting the contents

7) Remove excess liquid by grasping the plate firmly and **smartly** rapping the plate inverted on a clean paper towel at least 5 times
8) Fill each well with 400µl of **Wash Buffer** (Use Plate Washer setting R-D)
9) Repeat for a total of 6 washes.
10) After the last wash, **smartly** rap the inverted plate on a clean paper towel at least 10 times to remove excess **Wash Buffer**.

PLATE READER

Running the Program

Protocol

- Reading Parameters
  - Endpoint
  - Read mode: normal
  - Need to wavelengths, 490nm and 690nm
- Plate Layout
  - Standard: put units and values of concentrations from kit book
- Curve
  - Axis: extrapolation factor **3.0**
  - Curve Fit: 4 parameter
  - **This kit does not use blanks. The standard 8 should be set to blanks on the plate layout so the computer will automatically correct the Delta OD. Also use Curve interpretation to interpret Delta OD. Use Protocol R and D TNF-a (refer back to the TNF-a manual and the plate reader manual for instructions)**

Reading

- System: Administrator login: Admin
- Display: curves, allows you to look at your standard curve

Save, export

DETERMINATION OF ASSAY VALUES – STEP BY STEP

After performing the assay, you should have three concentration values for each sample ran; this would be your sample ran in triplicate. These values should be exported into an Excel document for easy interpretation. **If any values are above the extrapolation of the standard curve, you need to dilute and re-run those samples**. Open the excel file and arrange the data as suited for your needs. Below is an example of triplicate data for a single subject under 5 different experimental treatment conditions (A-E)

Subject 1	A	B	C	D	E
	0.8513	3.2736	6.2609	11.307	11.11
	0.9889	3.4701	6.8751	12.978	12.349
	0.753	3.76	8.2164	14.216	13.719

The next step is to identify the mean and standard deviation (SD) of each conditions triplicate values.

Subject 1	A	B	C	D	E
	0.8513	3.2736	6.2609	11.307	11.11
	0.9889	3.4701	6.8751	12.978	12.349
	0.753	3.76	8.2164	14.216	13.719
MEAN	0.8644	3.501233	7.117467	12.83367	12.39267
SD	0.118494	0.24469	1.000026	1.459861	1.305048

With the mean and standard deviation you can calculate the coefficient of variation (CV% = SD/mean*100) as depicted below.

Subject 1	A	B	C	D	E
	0.8513	3.2736	6.2609	11.307	11.11
	0.9889	3.4701	6.8751	12.978	12.349
	0.753	3.76	8.2164	14.216	13.719
MEAN	0.8644	3.501233	7.117467	12.83367	12.39267
SD	0.118494	0.24469	1.000026	1.459861	1.305048
CV	13.7%	7.0%	14.1%	11.4%	10.5%

The next step will be to eliminate the outlier. The outlier is the single value that is farthest away from the other two values. An example of the eliminated outliers is seen below. Note that all the CV fall within 10%. **If the CV is greater than 20% after the outlier has been removed, you need to rerun the sample**. *The mean of the two values that are left over should be used to represent the data.*

Note: The coefficient of variation has to be below 20%. If the CV for all three triplicate values is within 20%, you should average all values for your data.

Subject 1	A	B	C	D	E
	0.8513	3.2736	6.2609	11.307	11.11
		3.4701	6.8751	12.978	12.349
	0.753	3.76			
MEAN	0.80215	3.501233	6.568	12.1425	11.7295
SD	0.069509	0.24469	0.434305	1.181575	0.876105
CV	8.70%	7.00%	6.60%	9.70%	7.50%

CURRICULUM VITAE

Ryan A. Harris, M.S.

893 Deer Run – Ellettsville, Indiana 47429
Tel: 812.876.8931
Email: Harrisra@Indiana.edu

OFFICE
Indiana University
Clinical Exercise Physiology Laboratory
1025 East 7th St.
School of HPER, Room 070
Bloomington, IN. 47404
Tel: 812.855.7556

PERSONAL INFORMATION

Date of Birth: July 3, 1977
Place of Birth: Panorama City, California
Citizenship: United States
Marital Status: Married (Staci) and daughter (Kaelyn)
Social Security Number: xxx-xx-xxxx
Hobbies: Golf, soccer, basketball, physical fitness/exercise

EDUCATION

Doctor of Philosophy (Ph.D.) in Human Performance; Clinical Exercise Physiology
Indiana University, Bloomington, Indiana – In Progress

Dissertation: Interaction of exercise intensity on TNF-alpha, IL-6, and endothelial function.

Advisor: Janet P. Wallace, Department of Kinesiology, Clinical Exercise Physiology

Anticipated Graduation Date: May 2007

Master of Science (M.S.) Awarded in Kinesiology; Exercise Physiology
California State University Hayward, Hayward, California – March 2002

Bachelor of Science (B.S.) Awarded in Kinesiology; Fitness, Nutrition, and Health
San Diego State University, San Diego, California – December 1999

EMPLOYMENT

Associate Instructor**,** Indiana University	August 2003 – Present
Clinical Exercise Supervisor, Indiana University	August 2003 – December 2006
Owner/Developer, Bandercise Workout System www.Bandercise.com	June 2003 – Present
Exercise Physiologist**,** Valley Care Health System	November 2000 – June 2003
Personal Fitness Consultant**,** Bally's Total Fitness	April 1997 – March 1999

RESEARCH INTERESTS / OBJECTIVES

My present research interests involve cardiovascular disease and its co-morbidities. I am interested in the mono-layer of cells that lines all blood vessels, the endothelium. Specifically, I am interested in studying the endothelial response to an acute interventional paradigm that includes the: 1) dose response of exercise and endothelial function, 2) determination of the relationship between acute and chronic effects of exercise on endothelial function, 3) investigation of the relationship between blood pressure and endothelial function, 4) determination of responders and non-responders to exercise, 5) determination of the effect of static exercise on endothelial function, 6) identification of the exercise mechanism responsible for improvement of endothelial function in clinical populations, and 7) investigation of the postprandial endothelial response to a high fat meal.

Endothelial dysfunction is observed in many clinical populations. It is my objective to identify possible physiological mechanisms associated with the improvement of endothelial function which include, but are not limited to 1) inflammation, 2) oxidative stress, 3) and insulin resistance. In order to achieve my main objective, I plan to collaborate and establish a working relationship with other physiological and biochemical research scientist in the field.

UNIQUE RESEARCH SKILLS

- SPSS statistical program
- Ultrasonic measurement of endothelial function via flow-mediated dilation (FMD)
- Angiocath venipuncture placement and blood draw
- EKG interpretation
- Ambulatory blood pressure monitoring
- Biochemical assay analysis

MANUSCRIPTS IN PROGRESS

Harris, R.A., J. Padilla, J.P. Wallace. Time course of FMD following acute exercise in men. Target Journal: *Medicine and Science in Sports and Exercise.*

Padilla, J., **R.A. Harris**, J.P. Wallace. Characterization of post exercise induced shear stress. Target Journal: *Vascular Medicine.*

MANUSCRIPTS UNDER REVIEW

Harris, R.A., J. Padilla, K.P. Hanlon, L.D. Rink, J.P. Wallace. The interaction of IL-6 and TNF-alpha on the FMD response to acute exercise in overweight active and inactive men. Target Journal: *Circulation.*

Harris, R.A., J. Padilla, L.D. Rink, J.P. Wallace. Reproducibility of the Flow-Mediated Dilation Response to Acute Exercise in Overweight Men. Target Journal: *Ultrasound in Medicine and Biology*.

REFEREED PUBLISHED MANUSCRIPTS

Harris, R.A., D. Koceja. Comparison of electrically and mechanically induced H-reflex depression. *Electromyogr Clin Neurophysiol*. 46(7-8): 2006.

Padilla, J., **R.A. Harris**, A.D. Fly, L.D. Rink, J.P. Wallace. The effect of acute exercise on endothelial function following a high-fat meal. Eur. J. Appl. Physiol. 98(3): 256-262, 2006.

Padilla, J., **R.A. Harris**, A.D. Fly, L.D. Rink, J.P. Wallace. A comparison between active and reactive hyperaemia induced brachial artery vasodilation. *Clin. Sci.* 110(3): 387-392, 2006.

Harris, R. A., J. Padilla, L. D. Rink, J.P. Wallace. Variability of flow mediated dilation measurements with reactive hyperemia. *Vasc. Med.* 11(1):1-6, 2006.

NON-DATA BASED PUBLICATIONS

Harris, R. A., J Padilla. Endothelial dependent dilation and long-term exercise training. *Med. Sci. Sports Exerc.*, 38(7):1362, 2006.

COMPETITIVE PUBLISHED ABSTRACTS

Harris, R.A., K. Kitono, C.T. Robertson, D.K. Koceja. Electrically and Mechanically Induced Depression of the Soleus H-Reflex at Different Stimulus Intensities. *Med. Sci. Sports Exerc.* 38(5):S523, 2006.

Padilla, J., **R.A. Harris**, A.D. Fly, L.D. Rink, J.P. Wallace. A Comparison between Active and Reactive Hyperemia Induced Brachial Artery Vasodilation. *Med. Sci. Sports Exerc.* 38(5):S196, 2006.

Harris, R.A., J. Padilla, and J. P. Wallace. The effect of repetitive reactive hyperemia on flow-mediated dilation measurements. *Med. Sci. Sports Exerc.* 37(5):S221, 2005.

Padilla, J., **R.A. Harris**, and J. P. Wallace. Variation of flow-mediated dilation during morning hours. *Med. Sci. Sports Exerc.* 37(5):S221, 2005.

Harris, R.A., Padilla, J., Park, S., Wallace, J.P. Blood pressure reduction following physical activity: A case study approach. *Med. Sci. Sports Exerc.* 36(5):S251, 2004.

Padilla, J. Park, S., **Harris, R.A.**, Wallace, J.P. Ambulatory blood pressure response following free-living physical activity in pre- and hypertensive adults. *Med. Sci. Sports Exerc.* 36(5):S251, 2004.

Wallace, J.P. Padilla, J., Park, S., **Harris, R.A**. What is the adherence to free-living physical activity? *Med. Sci. Sports Exerc.* 36(5):S64, 2004.

INVITED PRESENTATIONS

Harris, R.A. The Influence of Diet and Exercise on Endothelial Function: A Series of Investigations. University of California, San Diego. La Jolla, California. March 2007.

Harris, R.A. The Influence of Diet and Exercise on Endothelial Function: A Series of Investigations. The Scripps Research Institute. La Jolla, California. November 2006.

Harris, R.A. Arterial Health and Exercise. *Kiwanis Club of Bloomington.* Bloomington, Indiana. September 2006.

Harris, R.A. Research Grant Winners Symposium. *Graduate and Professional Student Organization*. Indiana University, Indiana. February 2006.

Harris, R.A. Wellness Related Stress Management Break Out Sessions. *Cornerstone Information Systems*. Stone Mountain, Georgia. November 2004.

GRANT PROPOSALS

Pending

- HPER Travel Grant in Aid 2007
  Reproducibility of FMD Following Acute Exercise in Overweight Men

Funded

- HPER Graduate Student Research Grant in Aid Fall 2006 $600
  The Interaction of TNF-alpha, IL-6, and Acute Exercise on Endothelial Function in Overweight Men

- Research University Graduate School Dissertation Grant in Aid Fall 2006 $1000
  The Interaction of TNF-alpha, IL-6, and Acute Exercise on Endothelial Function in Overweight Men

- HPER Graduate Student Research Grant in Aid Spring 2006 $800
  Interaction of Exercise Intensity, TNF-alpha, and IL-6 on Endothelial Function

- Gatorade Sports Science Institute Student Grant 2005 $2300
  Interaction of Exercise Intensity, TNF-alpha, and IL-6 on Endothelial Function

- HPER Travel Grant in Aid 2005 $400
  The Effect of Repetitive Reactive Hyperemia on Brachial Artery Flow Mediated Dilation Measurements

-Graduate and Professional Student Organization Research Grant 2005 $200
  The Effect of Repetitive Reactive Hyperemia on Brachial Artery Flow Mediated Dilation Measurements

-HPER Travel Grant in Aid 2004 $400
  Blood Pressure Reduction Following Physical Activity: A Case Study Approach

Not Funded

- HPER Graduate Student Grant in Aid 2005
- American Heart Association Pre-Doctoral Fellowship 2005
- Graduate and Professional Student Organization Research Grant 2006

In Preparation

-HPER Graduate Student Research Grant in Aid Fall 2006
  Interaction of Exercise Intensity, TNF-alpha, and IL-6 on Endothelial Function

PROFESSIONAL AFFILIATIONS/CERTIFICATIONS

ACSM Exercise Specialist Professional Certification	2005 – Present
American College of Sports Medicine	2003 – Present
CPR Health Care Provider	2001 – Present
CPR Instructor	2001 – 2005

PROFESSIONAL/RESEARCH MEETINGS ATTENDED

ACSM Integrated Physiology, Indianapolis	2006
ACSM National Meeting, Tennessee	2005
ACSM National Meeting, Indianapolis	2004
ACSM National Meeting, San Francisco	2003
ACSM National Meeting, St Louis	2002

TEACHING RESPONSIBILITIES

Indiana University, Bloomington, Indiana

Graduate
K561 Clinical Exercise Physiology Laboratory
- Body composition (hydrostatic weighing and skinfolds)
- Heart rate, blood pressure, and double product
- Heart rate/VO_2 relationship
- EKG
- Spirometry simulating disease populations
- Ventilation
- Cardiac Output: Supine vs. upright

K567 Exercise Specialist Practicum
- Exercise counseling
- Body composition (hydrostatic weighing and skinfolds)
- EKG
- Pulmonary function
- Exercise capacity/ Oxygen consumption

Undergraduate
P409 Exercise Physiology Laboratory
- Muscular Strength
- Anaerobic power
- Predicted max oxygen consumption
- Ventilatory/Anaerobic thresholds
- Resting metabolic rate
- Maximal oxygen consumption

- Blood pressure
- Pulmonary function
- Body composition (hydrostatic weighing and skinfolds)

E119 Personal Fitness Lecture
- Understanding health related fitness and wellness
- Cardiorespiratory exercise prescription
- Improving muscular strength and endurance
- Nutrition, health, and fitness
- Prevention of cardiovascular disease
- Stress management

E119 Personal Fitness Laboratory
- Cardiorespiratory endurance fitness test
- Cardiorespiratory endurance program development
- Strength and endurance fitness testing
- Muscular strength and endurance program development
- Flexibility fitness test
- Flexibility program development

SUPERVISION OF GRADUATE STUDENT CURRICULUM

Clinical Exercise Physiology Masters Student Curriculum
- EKG
- Body Composition (skin folds and hydrostatic weighing)
- Pulmonary Function
- Metabolic Exercise Testing
- Exercise Prescription Counseling
- Adult Fitness Program Supervision

SERVICE

Departmental
- Guest lecture P420 Exercise Leadership for Special Populations (Spring 2007)
  Topic(s): Obesity and Exercise, Cardiovascular disease and Exercise part I and II.

- Guest lecture K639 Biochemistry of Exercise (Spring 2007)
  Topic: Angiocath placement

- Guest lecture N317 Diet, Disease, and Fitness (Spring 2006)
  Topic: Influence of diet on cardiovascular disease

- Supervise clients of the Adult Fitness Program (2003 – 2006)

- Guest lecture K561 Clinical Exercise Physiology (2004 – 2005)
  Topic: Endothelial function

- Guest lecture K562 Exercise Prescription in Health and Disease (2004 – 2006)
  Topic(s): Body composition, Pulmonary function, Blood values

Journal Referee
(JCR 2005 impact factor in italics)
- American Journal of Physiology: Heart and Circulatory Physiology (Feb. 2007) *3.56*

REFERENCES

Dr. Janet P. Wallace, Professor
Indiana University School of Health Physical Education and Recreation, Department of Kinesiology, HPER 070, 1025 East 7th Street, Bloomington, Indiana, 47401
Phone: 812.855.6384
Fax: 812.855.8179
Email: WallaceJ@Indiana.edu

Dr. David M. Koceja, Professor; Associate Dean for Research
Indiana University School of Health Physical Education and Recreation, Department of Kinesiology and Program in Neuroscience, HPER 111, 1025 East 7th Street, Bloomington, Indiana, 47401
Phone: 812.855.7302
Fax: 812.855.4983
Email: Koceja@Indiana.edu

Julie Frey, MS, Registered Clinical Exercise Physiologist
Internal Medicine Associates, 550 Landmark Avenue, Bloomington, Indiana, 47403
Phone: 812.331.3404
Fax: 812.355.6916
Email: Jfrey@ima-md.com

- Additional references provided upon request -