ABSTRACT

EFFECTS OF PRIOR AEROBIC EXERCISE ON VASCULAR DYSFUNCTION INDUCED BY PROLONGED SITTING IN HEALTHY MEN

by Robert Moir Duguid

Prolonged sitting has been shown to impair vascular endothelial function (VEF) in healthy men. Conversely, acute aerobic exercise improves VEF. PURPOSE: The objective of this study was to examine whether acute aerobic exercise would prevent impairments in VEF during sitting. METHODS: Eleven healthy men [21.2 ± 0.6 y (mean ± SE)] participated in two randomized 3-hour sitting trials preceded by a single bout of treadmill exercise (45 min at 65% maximal oxygen consumption (VO₂max)) (EX) or 45 min of rest (REST). Superficial femoral artery flow-mediated dilation (FMD) was assessed after an overnight fast (Pre), 1-hour following EX (or REST) (Post), and at 1-hour intervals during a 3-hour uninterrupted sitting challenge. Two-way repeated-measures ANOVA and Bonferroni post-hoc tests were used to evaluate differences within and between groups. RESULTS: In the REST trial, femoral artery FMD declined from Pre (2.6 ± 0.5%) at 1, 2, and 3 hours of sitting (P<0.05) and resting shear rate decreased at 3 hours (P<0.05). Femoral artery FMD (2.7 ± 0.6%) and resting shear rate responses were unaffected (P≥0.09) when sitting was preceded by EX. CONCLUSION: A single bout of aerobic exercise prevents the decline in FMD induced by 3-hours of uninterrupted sitting in healthy men.
EFFECTS OF PRIOR AEROBIC EXERCISE ON VASCULAR DYSFUNCTION INDUCED BY PROLONGED SITTING IN HEALTHY MEN

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EFFECTS OF PRIOR AEROBIC EXERCISE ON VASCULAR DYSFUNCTION INDUCED BY PROLONGED SITTING IN HEALTHY MEN

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Chapter 1
Introduction

Background: Adults in the United States spend approximately 8 hours per day in sedentary behaviors, specifically reclining, lying down, and sitting [1] [2]. This is concerning as greater amounts of sedentary time are associated with an increased risk of cardiovascular disease (CVD) and all-cause mortality [3] [4] [5]. Recent findings suggest that the link between increased time spent sedentary and reduced cardiovascular health is attributable, at least in part, to direct detrimental effects of sitting on vascular endothelial function (VEF) [6] [7] [8] [9] [10]. Arterial flow mediated dilation (FMD), the dilatory response to a transient increase in blood flow (i.e., vascular shear stress), is a standard non-invasive technique for assessing conduit artery (i.e., brachial, femoral) VEF and reflects nitric oxide (NO)-dependent dilation [11] [12]. Epidemiological studies show that arterial FMD predicts the risk of future cardiovascular events [13] [14]. In 12 healthy men, superficial femoral artery (i.e., lower extremity) FMD, but not brachial artery FMD (i.e., upper extremity), and arterial shear stress are decreased during 3-hours of prolonged, uninterrupted sitting [6] [15]. Impairment in femoral artery FMD was prevented by breaking up prolonged sitting with hourly 5 minute bouts of light-intensity walking [6], suggesting that regular bouts of light physical activity counteract the detrimental effects of prolonged sitting on VEF. Furthermore, indirect evidence from these same investigators [16] suggest that decreases in vascular shear stress occurring during prolonged sitting impairs femoral artery FMD by increasing oxidative stress and disrupting NO status. Nitric oxide is the primary vasodilator released from the vascular endothelium in response to an increase in blood flow and exerts numerous protective activities. In contrast to NO, endothelin-1 (ET-1) is a vasoconstrictor molecule which contributes to the reduction in NO status often associated with impaired VEF [17].

A single bout of aerobic exercise increases arterial FMD by 23-87% at 1-hour post-exercise [18] [19] [20] and remains elevated for up to 16-hours post-exercise [21]. In addition to improving arterial FMD, vascular shear stress measured at the brachial artery remains elevated for 2-hours following a 45 minute bout of continuous, moderate- to high-intensity (50-75% peak oxygen consumption (VO2peak)) treadmill exercise in healthy men [22]. These data suggest that
post-exercise elevations in shear stress contribute to acute exercise-induced increases in arterial FMD.

**Problem Statement:** It is currently unknown if a single bout of aerobic exercise performed prior to prolonged sitting can prevent sitting-induced impairments in arterial FMD. Furthermore, studies have yet to elucidate the influence of prolonged sitting on systemic markers of oxidative stress and VEF (i.e., NO, ET-1) and if aerobic exercise preceding prolonged sitting exerts beneficial effects on these markers. A better understanding of the mechanisms underlying the impairment in VEF induced by prolonged sitting will address a critical gap in our existing knowledge.

**Purpose Statement and Hypotheses:** The primary purpose of this investigation is to determine the effect of an acute bout of moderate-intensity aerobic exercise on superficial femoral artery FMD responses to 3-hours of prolonged, uninterrupted sitting in young, healthy men. Furthermore, we sought to determine potential biological mechanisms by which acute aerobic exercise alters FMD responses to prolonged sitting by measuring systemic markers of oxidative stress (i.e., malondialdehyde (MDA)) and VEF (i.e., NO status (arginine (ARG) and asymmetric dimethylarginine (ADMA), ET-1). Compared to a non-exercise control trial, we hypothesized that a single bout of moderate-intensity aerobic exercise would attenuate impairments in femoral artery FMD by mitigating increases in systemic oxidative stress and ET-1 levels and reductions in NO status that are otherwise induced by a 3-hour uninterrupted sitting challenge.

**Significance of Study:** By demonstrating that a prior bout of aerobic exercise protects against the detrimental effects of prolonged sitting on VEF (and elucidating potential biological mechanisms), we will add significant new findings to the areas of inactivity and vascular health. Specifically, these anticipated findings are timely and of public health significance given that sedentary activities are prevalent in the United States [1] and are associated with adverse mortality outcomes [5] [4] [3]. Findings of the present study will further highlight the acute protective effects of exercise, and provide a foundation for future studies to evaluate various lifestyle factors (i.e., different exercise modalities, timing of exercise, dietary strategies) to attenuate the deleterious effects of sedentary behavior on vascular health. Results from this study will lead to future research to determine whether prolonged sitting-induced changes in VEF are
influenced by sex, age, training status, or chronic disease. Ultimately, we expect that the successful completion of these studies will facilitate translational messages that improve the public’s perception of physical activity/exercise, and decrease time spent in sedentary behaviors. Furthermore, findings from this study will be of interest to those who sit for extended periods (e.g., long-distance travelers, office workers).
Chapter 2
Review of Literature

Overview: Review of the pertinent literature on this topic is divided into four related categories: 1) research methodologies to assess VEF and factors that influence vascular homeostasis; 2) epidemiological data linking sedentary behaviors and adverse health outcomes; 3) acute exercise and VEF responses and; 4) changes in circulating markers of VEF in response to acute exercise and prolonged sitting.

Vascular Endothelial Function Can Be Measured Non-Invasively: The interior lining of the systemic vasculature (lumen) consists of a single layer of endothelial cells. Endothelial cells synthesize and release vasodilatory and vasoconstrictor substances in response to specific stimuli to maintain vascular homeostasis. Nitric oxide (NO) is the primary vasodilator released by the vascular endothelium and has been investigated in a number of studies in humans [23] [24]. Shear forces (i.e., blood flow) exerted on the endothelium stimulate the production of NO from endothelial cells [24]. Shear stress is a direct measure of the forces exerted on the endothelium, however shear rate is a less invasive and more commonly reported measure of shear stress [25]. Shear stress is also important in the regulation of NO levels by stimulating endothelial nitric oxide synthase (eNOS) [26] [27]. Disruptions in normal shear stress patterns contribute to endothelial injury and contribute to the development of CVD [28]. Figure 1 depicts the cellular pathway of shear-stress induced NO production in endothelial cells.
Figure 1. The below figure illustrates the mechanism by which shear stress leads to increased vasodilation. Specifically, changes in shear stress transiently deform endothelial cells, leading to an increase in endothelial nitric oxide (NO) production. NO synthesis causes the underlying smooth musculature of the artery to relax, resulting in vasodilation. Adapted from [12].

A variety of techniques can be used to assess VEF, with varying degrees of invasiveness. Common methods for evaluating VEF include peripheral artery FMD [12] [11], iontophoresis [29], and venous occlusion plethysmography [30]. Iontophoresis is the delivery of vasodilator substances like acetylcholine across the skin using a weak electrical current and measuring the changes in blood perfusion via laser Doppler [9]. Venous occlusion plethysmography measures changes in limb volume via a strain gage while arterial inflow is maintained and venous return is contained within the limb [9]. Our study utilized arterial FMD exclusively as the measure of VEF. Arterial FMD is measured non–invasively by high resolution ultrasonography. The most common measurement sites for FMD are the brachial artery [12] or the superficial femoral artery.
Both brachial and femoral artery FMD reflect nitric oxide (NO)-dependent dilation [11] [12] [31]. The arterial FMD technique utilizes an inflatable cuff applied to the limb distal to the ultrasound probe and set to a suprasystolic pressure (e.g., 200-250 mmHg) for a period of 5 minutes to induce temporary ischemia and dilation of downstream resistance vessels. Upon cuff release, a transient period of reactive hyperemia occurs which stimulates arterial dilation. Changes in blood velocity and arterial diameter relative to resting measurements are used to calculate reactive hyperemia-induced shear stress. Arterial FMD is a clinically useful measure that predicts risk of CVD events [32], and is often considered alongside traditional cardiovascular risk factors [14].

Sedentary Behavior is Associated with Adverse Cardiovascular Health: Abundant research has been conducted on the interaction between lifestyle factors and cardiovascular disease, with increasing attention on the impact of time spent sedentary on cardiovascular health. Sedentary behavior, defined as activities done while sitting or reclining at ≤1.5 times resting energy expenditure, is on the rise [2] [1] and comprises approximately 55% of waking hours for adults and children in the United States [1]. This translates to an average sitting time for American adults is ~8 h/d [1]. Epidemiological data suggest that sitting time is directly associated with CVD risk factors, including total and LDL-cholesterol, triglycerides, waist circumference, and blood pressure [33] [34]. Furthermore, a large prospective study of ~123,000 US adults found that time spent sitting (≥6 vs. <3 h/d) was independently associated with increased risk of all-cause and CVD mortality during the 14 y follow-up period, regardless of physical activity level [35]. Therefore, increased time spent sitting is a public health concern, particularly given that it is common to many modern professions and CVD remains the leading cause of mortality in the US with ~800,000 deaths/y [36] [4]. Thus, a need exists to evaluate novel lifestyle strategies to counteract the detrimental cardiovascular effects of prolonged sitting.

The mechanism by which increased sitting time augments CVD risk [33] [35] [34] is not well understood. Recent experimental findings suggest that the link between increased sitting time and reduced cardiovascular health is attributable, at least in part, to direct detrimental effects of increased sedentary behavior on the vasculature. In particular, prolonged sitting reduces muscular activity of the lower extremities which decreases leg blood flow, increases blood pooling in the calf, augments mean arterial pressure, increases blood viscosity, and deforms
arterial segments resulting in low vascular shear stress [9]. Oxidative stress reflects an imbalance between the production of reactive oxygen species and the body’s antioxidant defenses and is increased in the presence of numerous CVD risk factors [37]. Low vascular shear stress with prolonged sitting [6] [15] decreases the expression of endothelial NO synthase [38] which reduces NO bioavailability and increases oxidative stress [39] [9], potentially impairing VEF and increasing CVD risk.

Impaired VEF is thought to underlie many cardiovascular diseases [13]. Studies demonstrate that prolonged sitting impairs arterial FMD, perhaps explaining, in part, the relationship between increased daily sitting time and CVD risk [33] [34] [35]. Specifically, three hours of prolonged sitting decreased FMD of the superficial femoral artery in 11 healthy young men (mean age = 24 y) [6] [16]. A separate study in 11 healthy young men (mean age = 27 y) showed that prolonged sitting for six hours decreased popliteal artery FMD [8]. In both studies, brachial artery FMD was unaffected, suggesting that lower extremity FMD is selectively impaired by prolonged sitting in healthy men.

Recent research shows that the negative effects of sitting can be mitigated through specific interventions. For example, Thosar et al. had 12 young healthy men complete 5 minute bouts of low-intensity walking (2 mph) performed at hourly intervals during a 3 hour bout of sitting, and compared FMD in the superficial femoral artery to the same subjects just completing an uninterrupted 3 hour bout of sitting [6]. They found that 3-hours of uninterrupted sitting significantly decreased FMD, whereas breaks in sitting time prevented impairments in femoral artery FMD. The authors also observed reductions in mean shear rate following the uninterrupted sitting, while shear rate was preserved during the walking trial, indicating a likely mechanism behind FMD preservation. Similarly, the detrimental vascular effects of sitting on superficial femoral artery FMD are present in young girls after 3 hours of sitting, but these effects were mitigated by 10 minutes of moderate-intensity exercise performed at hourly intervals [7]. Additionally, the simple act of fidgeting has been shown to be effective in preserving popliteal artery FMD during prolonged sitting in healthy men [40]. One study showed that ingestion of the dietary antioxidant vitamin C (1,500 mg) preserved superficial femoral artery FMD impaired by three hours of sitting in healthy men [16]. These data [16] suggest that prolonged sitting increases oxidative stress, perhaps reducing NO bioavailability [9] [39] and impairing FMD.
Increases in shear stress are thought to underlie the vascular protective effects associated with exercise [22]. Additionally, studies reporting decreases in FMD with prolonged sitting often observe an accompanying decrease in shear rate. For example, Thosar et al. observed a decrease in superficial femoral artery shear rate following three hours of uninterrupted sitting in young healthy men [6]. In contrast, shear rate (as well as FMD) were preserved with the addition of breaks in sitting time. Restaino et al. found similar decreases in shear rate in the popliteal artery at 2, 4 and 6 hours of sitting [8]. In addition to preserving FMD, fidgeting was also effective at increasing popliteal artery shear rate, compared to the control leg in 11 young, healthy subjects following 3 hours of sitting [40]. Sitting also selectively impairs lower body shear rate. Thosar et al. found that superficial femoral artery shear rate, but not brachial artery shear rate, decreased during 3 hours of sitting in young health men [15]. Given the unique characteristics of sitting, as well as the propensity for sitting to be the sedentary action of choice for most adults in the United States, more direct investigation into the impact of sitting on cardiovascular health is warranted. Since physical activity has been shown to be an effective intervention for improving cardiovascular health, exercise may prove to be an effective tool for preserving VEF, measured by superficial femoral artery FMD.

**Acute Exercise Improves Arterial Flow Mediated Dilation:** Physical activity is an effective lifestyle intervention to lower CVD risk [41] and many studies have been conducted to examine the efficacy of chronic exercise training and acute exercise on VEF. Indeed, studies show that a single bout of aerobic exercise increases both brachial and femoral artery FMD by 23-87% at 1-hour post-exercise [18] [19] [20] and brachial artery FMD remains elevated for up to 16-hours post-aerobic exercise [21]. In addition to improving arterial FMD, vascular shear stress measured at the brachial artery remains elevated for 2 hours following a 45 min bout of continuous, moderate- to high-intensity (50-75% peak oxygen consumption (VO2peak)) treadmill exercise in healthy men [22]. Interestingly, increases in arterial FMD do not appear to be limited to vascular tissue local to the working musculature, but instead exercise has a systemic effect on FMD. For instance, Totosy de Zepetnek et al. found that 9 minutes of upper body arm crank exercise increased superficial femoral artery FMD in healthy men [20]. These data suggest that exercise-induced elevations in shear stress contribute to increases in arterial FMD observed following acute aerobic exercise. It is currently unknown if improvements in systemic vascular
reactivity following acute aerobic exercise will persist during a prolonged sitting challenge shown previously to impair VEF [16].

**Acute Exercise Influences Circulating Measures of VEF:** Arterial FMD reflects nitric oxide (NO)-dependent dilation [11] [12] [31]. Nitric oxide released from the vascular endothelium cannot be directly measured in vivo, so analysis is commonly performed by collecting blood samples and measuring metabolites of NO. Ideally, samples would be collected locally in the blood vessel of interest (i.e., brachial or femoral artery), as this might better reflect changes in the local metabolic environment. Chronic exercise upregulates nitric oxide synthase (NOS) activity in rats [42]. In humans, acute aerobic exercise increases the bioavailability of NO when measured by quantitative determination of total nitrite [43]. Likewise, increases in forearm blood flow are abolished with the addition of NG-monomethyl-L-arginin (L-NMMA), an inhibitor of nitric oxide synthase, indicating that the increases in blood flow due to exercise are accomplished via an increase in nitric oxide bioavailability [44]. Aerobic exercise increases NOS expression (and consequently, NO) in humans with stable CVD [23]. Additionally, Zembron-Lancy et al. [45] found that increases in plasma NO were elevated above pre-exercise levels only when an eccentric component (downhill running at 65 % VO2max for 15 minutes) was present in the exercise intervention. In subjects with CVD, regular physical activity (30 minutes/day on a row ergometer and 30 minutes/day on a cycle ergometer for 4 weeks) increased the expression of NOS (measured via antibody detection in a tissue sample), suggesting improved ability to synthesize NO [23]. In contrast, high-intensity continuous aerobic exercise is related to dysfunction of eNOS-NO coupling, resulting in increased production of reactive oxygen species [46].

Reactive oxygen species (i.e., oxidative stress) have been suggested to decrease NO bioavailability via several pathways, including the inhibition of endothelial NOS and decreasing arginine availability [47] [48]. Arginine, the amino acid required for NO biosynthesis, and asymmetric dimethylarginine ADMA, an endogenously produced competitive inhibitor of NOS are measured in plasma and used to indirectly examine NO bioavailability. Briefly, oxidative stress inhibits dimethylarginine dimethylaminohydrolase, and enzyme which degrades ADMA [47]. The ratio of plasma ADMA:arginine has been previously used to indirectly evaluate NO bioavailability in the endothelium following a postprandial challenge [47] [49], but has not been
previously investigated relative to prolonged sitting. Data in healthy men [16] suggest that prolonged sitting increases oxidative stress, perhaps reducing NO bioavailability [9] [39] and contributing to impairments in femoral artery FMD. To our knowledge, no studies have reported changes in circulating markers of VEF in response to prolonged sitting. Previous prolonged sitting studies have cited logistics and subject discomfort as prohibiting direct lower body blood sampling [16]. In contrast to NO, endothelin-1 (ET-1) is a vasoconstrictor molecule which contributes to the reduction in NO status often associated with impaired VEF [17]. Endothelin-1 (ET-1) is released by the vascular endothelium and has been shown to attenuate basal leg flow and popliteal artery FMD in seven young, healthy subjects. [50]. Indeed, blood levels of ET-1 are inversely associated with FMD [51] [52]. Furthermore, aerobic exercise increases NO and decreases ET-1 levels [51]. In the present study, we sought to investigate physiological mechanisms underlying expected changes in VEF during prolonged sitting by collecting blood samples from a forearm vein and analyzing plasma specimens for markers of oxidative stress [malondialdehyde (MDA)], NO status [arginine and ADMA], and ET-1. Given that femoral artery FMD is impaired during prolonged sitting [7] [8] [6] [15] [16], and prior studies show that acute aerobic exercise transiently increases arterial FMD [18] [19] [20], this study compared the efficacy of performing acute, moderate-intensity aerobic exercise on VEF responses to prolonged sitting.

**Purpose Statement and Hypotheses:** The primary purpose of this investigation is to determine the effect of an acute bout of moderate-intensity aerobic exercise on femoral artery FMD responses to 3 hours of prolonged sitting in young, healthy men. Furthermore, we sought to determine potential biological mechanisms by which acute aerobic exercise alters FMD responses to prolonged sitting by measuring systemic markers of oxidative stress and VEF (i.e., NO status, ET-1). Compared to a non-exercise control trial, we hypothesized that a single bout of moderate-intensity aerobic exercise would attenuate impairments in femoral artery FMD by mitigating increases in systemic oxidative stress and ET-1 levels and reductions in NO status that are otherwise induced by a 3 hour uninterrupted sitting challenge.
Chapter 3
Methodology

Study Design: The protocol for this study was approved by the Institutional Review Board at Miami University and written informed consent (Appendix A) was obtained from all participants before enrolling. Healthy men completed a randomized, cross-over study separated by ≥1 week. Following a phone screening to determine eligibility (Appendix B), a screening visit was conducted to obtain a fasting blood sample (≥8-hours) and measure maximal oxygen consumption (VO$_{2\text{max}}$). Participants were instructed to avoid strenuous exercise for 48-hours preceding the screening visit and each trial visit. Approximately 1 week following the screening visit, participants reported to our laboratory in the fasted state for femoral artery FMD assessment and blood collection. Immediately thereafter, they performed 45 minutes of moderate-intensity treadmill exercise (EX) or 45 minutes of quiet rest (REST) as a control condition. Superficial femoral artery FMD and venous blood samples were obtained 1-hour after EX (or REST) and then hourly during 3-hours of uninterrupted sitting. The two prolonged sitting trials occurred at approximately the same time of day (≤1-hour).

Participants: Healthy men (n = 11) were enrolled on the basis of age (18-30y), non-smoking status, and body mass index (BMI) <30 kg/m$^2$. Participants were not taking medications for chronic disease or dietary supplements. Participants were instructed to maintain their current level of physical activity throughout the study duration. Additionally, participants completed a food log for 1-day prior to their first testing trial and instructed to replicate their dietary intake the day prior to their second testing trial. Food records were assessed for nutrient intake using Food Processor software (ESHA Research, Salem, OR).

Screening Visit: After explaining the study procedures and obtaining written informed consent, participants completed medical history and physical activity questionnaires (Appendix C). Participants were instructed on how to complete a food record by study personnel (Appendix D), followed by measurement of height, weight, and waist circumference using standard procedures. Next, body composition was measured by bioelectrical impedance analysis (InBody 770, Cerritos, CA, USA) and resting heart rate and blood pressure were recorded two times using an automated blood pressure monitor (Omron HEM907XL, Bonnockburn, IL, USA) following five minutes of seated rest. A fasted blood sample was obtained from an antecubital vein for the
determination of plasma glucose and triglyceride concentrations. Finally, participants performed a graded exercise test on a motor driven treadmill to determine maximal oxygen consumption (VO$_2$max) (Appendix E). Expired air was collected and analyzed using a calibrated Parvomedics True One 2400 Metabolic System (ParvoMedics, Sandy, UT, USA).

**Testing Trials:** Approximately 1-week following the screening visit, participants returned to the laboratory after an overnight fast and having abstained from exercise for >48 hours. Participants were seated for at least 10 minutes in a comfortable chair with back support before the assessment of baseline (Pre) femoral artery FMD (Figure 2). Next, a blood sample was obtained from an antecubital vein, after which participants were provided with a standardized snack (Nature Valley oats and honey granola bar; 190 kcals; 29/7/3g (carb/fat/pro)). Consumption of the standardized snack was intended to prevent hypoglycemia and provide a similar postprandial milieu between testing trials. Following 30 minutes of quiet rest, participants completed a 45 minutes bout of treadmill exercise at a speed and grade corresponding to 65% VO$_2$max or 45 minutes of quiet rest. During the REST trial, participants rested quietly in the laboratory. The treadmill exercise bout used in the present study was similar in intensity and duration to prior studies showing greater 3-hour post-exercise arterial shear rate responses following high-intensity (75% VO$_2$max) compared to moderate- (50% VO$_2$max) and low-intensity (25% VO$_2$max) treadmill walking in healthy, middle-aged men [22]. Expired air was collected and analyzed using a calibrated Parvomedics True One 2400 Metabolic System (ParvoMedics, Sandy, UT, USA) at 5 minute intervals during the first 15 minutes of exercise to confirm that participants were within ±5% of their individual VO$_2$max. If the subject’s intensity was not within that window, the treadmill speed and/or grade were adjusted until the subject was at the correct intensity. After the subject demonstrated a stable VO$_2$ within the desired range, the mouthpiece was removed for the duration of the 45 minute exercise session. Water was consumed *ad libitum* throughout exercise. Thirty minutes following the completion of EX (or REST), a second snack was provided which was identical to that consumed at Pre.
Figure 2. Timeline of study measures. Superficial femoral artery FMD was assessed and blood obtained after an overnight fast (Pre), 1-hr after 45 minutes of aerobic exercise (EX) or quiet rest (REST), and at 1-hr intervals for 3-hr of uninterrupted sitting. Blood samples were obtained from an antecubital vein immediately following the completion of FMD at each time point. FMD = flow mediated dilation.

Flow Mediated Dilation: Superficial femoral artery FMD was assessed by high-frequency ultrasonographic imaging at each time point by a trained technician. In brief, the rapid inflation cuff was placed on the right lower thigh approximately 7 cm proximal to the knee joint and the superficial femoral artery was imaged longitudinally 7-10 cm below the inguinal crease using a 5- to 12-MHz multi-frequency linear array transducer connected to a high-resolution ultrasound (uSmart 3300; Terason, Burlington, MA, USA) (Figure 3). The participants’ skin was marked
and the distance from the inguinal crease to the proximal edge of the transducer was recorded to ensure similar placement of the transducer for subsequent FMD measures within and between trials, respectively. Pre-occlusion end-diastolic femoral artery diameter and continuous Doppler velocity was assessed simultaneously for 1 min. The thigh cuff was then inflated to 250 mmHg for 5 min using a rapid cuff inflator (Hokanson E20, Bellevue, WA, USA), and then rapidly released. Recordings of arterial diameter and blood velocity were obtained 1 min prior to cuff deflation and for 3 min thereafter at 10 frames/s (Camtasia Studio, TechSmith Corporation, Okemos, MI, USA). Offline analyses were performed using automated edge-detection software with end-diastolic gating (Medical Imaging Applications, Iowa City, IA, USA). All vascular measurements and analyses were performed by the same technician. From end-diastolic synchronized diameter (D; mm) and velocity data (V; m • s⁻¹), shear rate was calculated as 4(V)/D (28). Shear rate area under the curve (AUC_{SR}) was calculated from the time of cuff release until the time of maximal post-occlusion diameter to determine the hyperemic stimulus responsible for FMD (29).

Figure 3. Prolonged Sitting Protocol Used in the Present Study.

**Blood Processing and Analyses:** Blood was collected into evacuated tubes containing EDTA and lithium heparin. Whole blood was centrifuged (3000 rpm, 4°C, 15 min), plasma separated,
and archived at -80°C until study end. Plasma triglycerides (screening visit only) and glucose (screening and trial visits) were measured using commercially available clinical assays (Pointe Scientific, Canton, MI, USA) on a microplate reader (BioTek Instruments, Synergy HT, Winooski, VT, USA). Plasma ET-1 was measured using an ELISA kit in accordance with the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA).

HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA) as were the following chemicals: ascorbic acid, butylated hydroxytoluene (BHT), DTPA, glacial acetic acid, methylmonoarginine, o-phthalaldehyde, PCA, potassium hydroxide, sodium hydroxide, sodium acetate, and trichloroacetic acid (TCA). γ-Glutamylglutamate was purchased from Acros Organics (Fair Lawn, NJ, USA). Dansyl chloride and thiobarbituric acid (TBA) were from Sigma (St. Louis, MO, USA) and Q-12 ion pairing reagent was from Regis (Morton Grove, IL, USA).

Plasma malondialdehyde (MDA), a measure of lipid peroxidation, was measured by HPLC-FL as described [47], with minor modification. Briefly, 200μL heparin plasma was mixed with 20μL 0.2% BHT and 100μL 1N sodium hydroxide solution, and incubated (30 min at 60°C) before adding 1.0mL 10% TCA and then cooling on ice for 10 min. After centrifugation (1,100 x g, 4°C, 10 min), the supernatant was mixed with 100μL 0.6% (w:v) TBA, heated at 95°C for 30 min, and then rapidly cooled before extracting with 200μL butanol. Following centrifugation, the supernatant was injected onto a Shimadzu LC-20XR system (Shimadzu, Columbia, MD, USA) equipped with a RF-10AXL fluorescence detector set to 532/553 nm (excitation/emission). HPLC separation was performed isocratically at 0.8 mL/min on a C18 separation column (250 x 4.6 mm i.d., 5 mm; Phenomenex, Torrance, CA, USA) using 50:50 methanol and 25 mmol/L phosphate buffer (pH 6.5) as the mobile phase. Plasma MDA concentrations were quantified from standards prepared in parallel from tetramethoxypropane.

Plasma arginine and asymmetric dimethylarginine (ADMA) were measured as indirect indicators of NO status as described [47], with minor modification. Arginine and ADMA were extracted from heparin plasma (100 μL) by solid phase extraction on a polymeric cation-exchange column (Hypersep Retain-CX SPE column; 30 mg, 1 mL; Fisher Scientific, Pittsburgh, PA, USA) using ammonia:water:methanol (10:40:50, v:v:v). HPLC separation was performed isocratically at 1.3 mL/min on a Shimadzu LC-20ASXR system equipped with a RF-20AXL
fluorescence detector programmed to 340/455 nm (excitation/emission) and a Phenomenex Kinect XB-C18 column (50 x 3.0 mm i.d., 2.6 μm; phenomenex). O-phthalaldehyde-derivatives of arginine and ADMA were eluted from the column using 50mmol/L potassium phosphate buffer (pH 6.5) and 6.5% (v:v) acetonitrile as the mobile phase. After the peak of arginine eluted from the column, the gain setting was increased 16 fold at 3 min to enable the detection of ADMA. After the last peak of interested eluted, the column was washed with 50% acetonitrile for 2 min and the system was equilibrated for 2 min before the next injection. Analytes were quantified on the basis of peak area relative to internal standard (methylmonoarginine). The ratio of ADMA/ARG was calculated as an indirect index of NO bioavailability.

**Statistical Analyses:** Sample size was determined utilizing data from a previous cross-over study showing that 3-hours of uninterrupted sitting significantly reduced femoral artery FMD in 12 young healthy men [6]. Data [6] indicated that baseline femoral artery FMD responses did not differ between trials (4.6 ± 3.1%; mean ± SD) and decreased to 0.5 ± 0.9% at 1-hour of prolonged sitting. Our calculations indicated that a minimum of 6 participants would be needed in the present study in order to reject the null hypothesis with 80% power (P<0.05). Data were analyzed by SPSS. Prior to all analyses, data were assessed for normality using Shapiro-Wilk’s W-tests. Repeated measures ANOVA was used to evaluate differences due to treatment, time, and their interaction for the dependent variables. In the presence of significant effects, Bonferroni post hoc analyses were performed adjusting the P-value for the number of pairwise comparisons. A Student’s paired t-test was used to evaluate dietary intakes between trials. An α-level of P≤0.05 was considered statistically significant for all analyses.
Chapter 4

Results

Participants and Dietary Intakes: Eleven (n = 11) healthy men completed our study (Table 1). Participants reported engaging in moderate-intensity physical activity approximately 2 days/week (range = 0-5 days). Energy and nutrient intakes did not differ between trials (Table 2), indicating that participants had similar dietary intakes the day prior to each trial. Participants completed the 45 minutes of treadmill exercise at 65.6 ± 3.9% VO2max (range = 60.9-72.6%).

Femoral Artery Flow Mediated Dilation: Femoral artery FMD responses did not differ between trials at Pre. Significant main effect for time and trial were found for relative FMD (Figure 4). In the REST trial, FMD responses decreased at 1-3 hours of prolonged sitting compared to Pre (P<0.05). Adjusting the P-value for the number of pairwise comparisons (0.05/4 = 0.013) revealed that only the 2-hour time point was significantly lower (P<0.01) compared to Pre. Femoral artery FMD responses throughout the EX trial were unaffected (P≥0.33). Time-matched FMD responses were higher (P = 0.03) at 2-hours in the EX trial compared to the REST trial. Absolute FMD responses showed a similar pattern.

Pre-occlusion femoral artery diameters did not differ within or between trials (Table 3). Main and interaction effects were found for pre-occlusion shear rates (Figure 5). Compared to Pre, the increase in pre-occlusion shear rate at Post did not reach statistical significance (P = 0.09), likely due to the large variability. Compared to Pre, pre-occlusion shear rate decreased at 3-hours of prolonged sitting (P<0.05) in the REST trial but did not reach statistical significance after adjusting the P-value for the number of pairwise comparisons. Time-matched shear rate responses were higher (P = 0.01) at Post in the EX trial compared to the REST trial. Main effects for time and trial occurred for maximal post-occlusion diameters (Table 3). Compared to Pre, time-dependent decreases in maximal post-occlusion diameter occurred at 3-hours during the EX trial (P = 0.04) and at 2-3 hours during the REST trial (P≤0.02). Time-matched maximal post-occlusion diameter responses were higher at Post (P = 0.02) and at 2-hours (P = 0.04) in the EX trial compared to the REST trial. No significant pairwise comparisons were detected after adjusting the P-value. Shear rate area under the curve was unaffected within- and between-trials (Table 3).
**Plasma Glucose:** Plasma glucose did not differ between trials at Pre (Table 4). A significant main effect for time was found for plasma glucose. Compared to Pre, plasma glucose increased at Post ($P = 0.02$) by 12% and 15% in the EX and REST trial, respectively. Calculation of percent change scores for plasma glucose relative to Pre showed a similar pattern.

**Oxidative Stress and Nitric Oxide Status:** Plasma MDA, ARG, ADMA, and ADMA/ARG did not differ between trials at Pre (Table 4). A significant main effect for time and interaction effect were found for plasma MDA and ARG, respectively. No significant pairwise differences were detected.

**Endothelin-1:** Plasma ET-1 did not differ between trials at Pre (Table 4). A significant main effect for time was found for plasma ET-1. However, no significant pairwise differences were detected (all $P \geq 0.09$).
Chapter 5
Discussion

The purpose of this study was to investigate the effects of a single bout of moderate-intensity aerobic exercise on superficial femoral artery FMD responses to 3-hours of prolonged, uninterrupted sitting in healthy men. Additionally, we sought to determine if circulating markers associated with VEF (i.e., plasma MDA, ARG, ADMA, ET-1) were affected by acute exercise performed prior to prolonged sitting. Compared to a non-exercise control trial, we hypothesized that prior aerobic exercise would attenuate impairments in superficial femoral artery FMD by mitigating increases in systemic lipid peroxidation (i.e., MDA) and ET-1 levels and reductions in NO status (i.e., ARG, ADMA) that are otherwise induced by a 3-hour uninterrupted sitting challenge. To our knowledge, our study is the first to examine the effects of exercise performed prior to prolonged sitting, as well as the first to evaluate changes in circulating markers related to VEF in response to prolonged sitting. The primary finding of our study was that an acute bout of aerobic exercise prevented impairments in VEF induced by prolonged sitting. Specifically, we showed that superficial femoral artery FMD responses to 3-hours of prolonged, uninterrupted sitting are unaffected when preceded by 45 minutes of moderate-intensity aerobic exercise whereas sitting decreased FMD in the non-exercise trial. Thus, these data support that acute aerobic exercise prevents vascular dysfunction otherwise impaired by prolonged sitting. The decrease in lower-extremity FMD did not coincide with systemic changes in MDA, ARG, ADMA, or ET-1, suggesting that impairments in VEF with prolonged sitting are not attributed to higher oxidative stress responses that decrease NO status [53] and increase ET-1 [54]. Furthermore, the vasoprotective activities of acute aerobic exercise performed prior to prolonged sitting were not attributed to systemic changes in lipid peroxidation or VEF markers.

Sedentary behavior is prevalent [1] [2], with American adults sitting on average approximately 8-hours per day [1]. This is concerning not only because sedentary behavior is common in modern society but epidemiological data suggest that time spent sedentary is directly associated with CVD risk factors [1] [3] [4] [33] [34] and CVD and all-cause mortality [5] [35]. Recent studies attribute the link between increased time spent sedentary and reduced cardiovascular health to the detrimental effects of sitting on VEF [7] [8] [6] [16]. Indeed, prior studies have shown that lower body FMD is decreased by prolonged sitting. In 12 healthy men,
Thosar et al. [6] showed that 3-hours of uninterrupted sitting decreased superficial femoral artery FMD, with maximal impairment occurring at the 1-hour time point. A separate study in 11 healthy men reported that 6-hours of uninterrupted sitting decreased popliteal artery FMD [8]. Brachial artery FMD was unaffected in these participants [8] [15], likely due to participants using their arms to perform light work (e.g., reading, computer work) during sitting, supporting that lower body FMD is selectively impaired by prolonged bouts of sitting varying in duration. Furthermore, 3-hours of uninterrupted sitting was shown to impair superficial femoral artery FMD in young girls [7]. Consistent with prior studies [6] [15] [16], we showed that 3-hours of prolonged, uninterrupted sitting impaired superficial femoral artery FMD in healthy men.

Studies showing reductions in lower body FMD with prolonged sitting have reported concomitant decreases in resting mean shear rate [6] [8]. In our control trial, we observed a steady, albeit non-significant, decline in resting mean shear rate during 3-hours of uninterrupted sitting. Cumulatively, data from the present study and from others [6] [8] suggest that uninterrupted sitting induces a state of low arterial shear stress that contributes to reductions in FMD. Previous studies have employed several novel strategies aimed at preventing reductions in shear stress and lower body FMD induced by prolonged sitting. Thosar et al. [6] incorporated light activity breaks (5 minutes of treadmill walking at 2 mph) at regular intervals during 3-hours of sitting in 12 healthy men and observed that breaks prevented sitting-induced reductions in both resting mean shear rate and superficial femoral artery FMD. Additionally, Restaino et al. [8] observed that 6-hours of uninterrupted sitting significantly decreased both resting mean shear rate and popliteal artery FMD in 11 healthy men. Six-hours of sitting was followed by a 10 minute walk at a self-selected pace, which was effective at restoring shear rate and FMD to pre-sit levels. A separate study in 10 healthy men investigated localized heating as a strategy to modify shear stress and FMD during prolonged sitting [55]. In this study [55], 10 healthy men sat for 3-hours with one foot submerged in a heated water bath maintained at 42° C to increase shear stress, while the opposite foot remained dry. Popliteal artery mean shear rate and FMD were reduced after 3-hours of sitting in the non-heated leg, whereas both mean shear rate and FMD in the heated leg were significantly higher compared to pre-sitting measures. Collectively, these data [8] [6] [55] support that reductions in shear stress with prolonged sitting impair lower-body VEF. Findings of our study in healthy men extend these data by showing that resting mean
shear rate of the superficial femoral artery was significantly higher 1-hour following aerobic exercise compared to the control trial, perhaps contributing to the preservation of FMD observed when acute exercise preceded prolonged sitting.

Thosar et al. [16] recently observed in 11 healthy men that decreases in resting mean shear rate and superficial femoral artery FMD induced by 3-hours of uninterrupted sitting was prevented by ingestion of 1,500 mg of the dietary antioxidant vitamin C, suggesting that increased oxidative stress induced by low vascular shear stress [9] [39] may contribute to the impairment in lower-body FMD during prolonged sitting [8] [6] [55] by reducing NO bioavailability [38]. In our cohort of healthy men, we did not observe changes in systemic oxidative stress (i.e., MDA) during 3-hours of uninterrupted sitting. Further, plasma levels of ARG and ADMA were unaffected by sitting, suggesting possible preservation of NO bioavailability. Collectively, our data suggest that 3-hours of uninterrupted sitting impairs superficial femoral artery FMD by an oxidative stress-independent mechanism. Additionally, plasma levels of the vasoconstrictor ET-1 were unaffected by prolonged sitting and acute exercise, likely due to the lack of change in oxidative stress in our participants [54]. Alternative mechanisms potentially explaining reductions in lower-body FMD with prolonged sitting have been suggested [16], including increases in blood viscosity, inflammation, and coagulation, and warrant investigation in future studies.

Limitations: Though our study was sufficiently powered to detect differences in superficial femoral artery FMD based on published data [6] and our sample size was similar to others [8] [6] [55], we may not have had sufficient power to detect differences in circulating measures of VEF. Young men were recruited for the study in order to compare study results to previous methodologies investigating prolonged sitting [8] [6] [55]. A recent study in young healthy adults found that popliteal artery FMD decreased in eight men in response to 3-hours of uninterrupted sitting, whereas FMD was unaffected in response to sitting in 12 pre-menopausal women who were tested during the same phase of their menstrual cycle [56]. Differences in FMD responses between sexes occurred despite comparable reductions in resting popliteal artery mean shear rate during 3-hours of uninterrupted sitting [56]. Additionally, we did not attempt to obtain blood samples from the leg based on difficulties reported by others [6]. We did not observe changes in plasma levels of MDA, ARG, ADMA, or ET-1, suggesting that systemic
alterations in oxidative stress, NO status, or vasoconstriction do not underlie decreases in superficial femoral artery FMD with prolonged sitting. However, our data could also indicate that blood samples collected from the arm are not reflective of perturbations occurring in the lower-body vasculature.

**Conclusion:** This study advances existing knowledge that sedentary behavior increases CVD risk [10] [33] [34] [35] and that prolonged, uninterrupted sitting impairs lower-body VEF [7] [8] [6] [16] [55] by showing that a prior bout of moderate-intensity aerobic exercise prevents impairments in superficial femoral artery FMD otherwise induced by 3-hours of prolonged sitting. This is of public health importance, consistent with epidemiological findings suggesting that increased daily sitting time predicts CVD and all-cause mortality [3] [4] [5] [35]. Three-hours of uninterrupted sitting impaired superficial femoral artery FMD without changing systemic markers related to VEF. Our data show that aerobic exercise is an effective lifestyle strategy to attenuate sitting-induced vascular endothelial dysfunction, a finding with practical implications for individuals whose occupations require long periods of sitting (e.g., long-distance travel, office workers). Future studies should be conducted to further explore the physiological mechanisms underlying sitting-induced impairment of VEF as well as evaluate various lifestyle factors (i.e., different exercise modalities, timing of exercise, dietary strategies) to attenuate the deleterious effects of prolonged sitting on vascular health. Additionally, future research is warranted to determine whether sitting-induced changes in VEF are influenced by age, sex, training status, or chronic disease.
TABLE 1. Participant characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.2 ± 1.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.2 ± 4.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.6 ± 3.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 1.0</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>17.0 ± 5.2</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>81.3 ± 8.7</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119.5 ± 10.7</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64.2 ± 10.0</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>62.0 ± 10.7</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>49.9 ± 5.1</td>
</tr>
<tr>
<td>Physical Activity (d/wk)</td>
<td>1.9 ± 1.6</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>78.6 ± 35.9</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>92.6 ± 9.1</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n = 11). Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; VO₂max, maximal oxygen consumption. Plasma concentrations for triglyceride and glucose.
### TABLE 2. Participants’ dietary intakes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EX</th>
<th>REST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Energy (kcal/d)</td>
<td>2475 ± 737</td>
<td>2384 ± 692</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>50.4 ± 13.5</td>
<td>50.6 ± 12.9</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>18.2 ± 5.5</td>
<td>18.3 ± 9.8</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32.3 ± 10.2</td>
<td>33.0 ± 9.9</td>
</tr>
<tr>
<td>Saturated Fat (g/d)</td>
<td>28.7 ± 23.2</td>
<td>28.4 ± 22.7</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>237.2 ± 174.3</td>
<td>207.3 ± 153.1</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 11. Dietary intakes were determined from food records for the 1-day preceding each intervention trial. Nutrient intakes were analyzed using Food Processor software (ESHA Research, Salem, OR, USA). There were no differences in dietary intakes between trials (Paired t-test, P≥0.50 for each dietary parameter).
TABLE 3. Superficial femoral artery responses in participants who completed the exercise (EX) and control (REST) trials.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EX</th>
<th>REST</th>
<th>Time (P-value)</th>
<th>Trial (P-value)</th>
<th>Time x Trial (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Occlusion Diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>6.74 ± 0.61</td>
<td>6.61 ± 0.63</td>
<td>0.29</td>
<td>0.28</td>
<td>0.04</td>
</tr>
<tr>
<td>Post</td>
<td>6.71 ± 0.58</td>
<td>6.54 ± 0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>6.65 ± 0.60</td>
<td>6.69 ± 0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>6.62 ± 0.57</td>
<td>6.58 ± 0.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>6.61 ± 0.58</td>
<td>6.58 ± 0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Occlusion Shear Rate (s⁻¹)</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Pre</td>
<td>74.3 ± 39.0</td>
<td>67.9 ± 25.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post *</td>
<td>115.8 ± 55.3</td>
<td>61.6 ± 13.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>70.2 ± 22.2</td>
<td>55.6 ± 18.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>60.2 ± 13.1</td>
<td>51.2 ± 13.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>59.9 ± 17.5</td>
<td>52.8 ± 15.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal Post-Occlusion Diameter (mm)</td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>Pre</td>
<td>6.92 ± 0.62</td>
<td>6.78 ± 0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>6.92 ± 0.57</td>
<td>6.66 ± 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>6.81 ± 0.57</td>
<td>6.76 ± 0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>6.77 ± 0.59</td>
<td>6.61 ± 0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data are mean ± SD, n = 11. Analyses were performed using 2-way RM ANOVA to determine main and interactive effects. Bonferroni’s post-test was used to evaluate pair-wise differences. * P≤0.01 between trials. No other significant (P≤0.01) pair-wise differences were detected. Abbreviations: AUC_{SR} = shear rate area under the curve. # P<0.01 between trials.
TABLE 4. Plasma concentrations of glucose, oxidative stress, nitric oxide status, and endothelin-1 in participants who completed the exercise (EX) and control (REST) trials.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EX</th>
<th>REST</th>
<th>Time (P-value)</th>
<th>Trial (P-value)</th>
<th>Time x Trial (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>&lt;0.01</td>
<td>0.78</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>96.1 ± 9.3</td>
<td>91.8 ± 7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>106.7 ± 14.9</td>
<td>105.3 ± 12.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>89.5 ± 6.4</td>
<td>92.7 ± 11.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>92.2 ± 10.8</td>
<td>90.5 ± 8.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>86.2 ± 13.9</td>
<td>91.9 ± 8.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>0.01</td>
<td>0.35</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.07 ± 0.27</td>
<td>1.06 ± 0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>1.16 ± 0.28</td>
<td>1.17 ± 0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>1.00 ± 0.14</td>
<td>0.95 ± 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>1.05 ± 0.11</td>
<td>1.00 ± 0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>1.06 ± 0.15</td>
<td>1.01 ± 0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADMA (nmol/L)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>493.6 ± 92.2</td>
<td>446.1 ± 57.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>479.2 ± 110.8</td>
<td>466.1 ± 149.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>435.2 ± 100.3</td>
<td>415.3 ± 143.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARG (μmol/L)</td>
<td>ADMA/ARG (nmol/μmol)</td>
<td>Endothelin-1 (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 h</td>
<td>3 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>472.3 ± 106.9</td>
<td>440.8 ± 105.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>443.8 ± 88.2</td>
<td>426.8 ± 99.3</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>4.11</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>71.1 ± 18.2</td>
<td>65.7 ± 13.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>72.1 ± 20.8</td>
<td>68.6 ± 14.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>66.3 ± 14.9</td>
<td>62.0 ± 15.0</td>
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<tr>
<td>2 h</td>
<td>61.4 ± 7.7</td>
<td>64.7 ± 9.3</td>
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<tr>
<td>3 h</td>
<td>62.7 ± 7.3</td>
<td>66.8 ± 9.2</td>
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<td></td>
<td></td>
<td>0.26</td>
<td>0.17</td>
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<tr>
<td>Pre</td>
<td>7.26 ± 2.07</td>
<td>6.98 ± 1.22</td>
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<tr>
<td>Post</td>
<td>6.83 ± 1.44</td>
<td>6.90 ± 1.98</td>
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<tr>
<td>1 h</td>
<td>6.76 ± 1.73</td>
<td>6.81 ± 1.92</td>
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<td>2 h</td>
<td>7.73 ± 1.60</td>
<td>6.82 ± 1.26</td>
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<tr>
<td>3 h</td>
<td>7.20 ± 1.78</td>
<td>6.46 ± 1.56</td>
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<td>&lt;0.01</td>
<td>0.21</td>
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<tr>
<td>Pre</td>
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<td>1.78 ± 0.52</td>
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<td>Post</td>
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<td>1.81 ± 0.55</td>
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Data are mean ± SD, n = 11. Analyses were performed using 2-way RM ANOVA to determine main and interactive effects. Bonferroni’s post-test was used to evaluate pair-wise differences. No significant (P≤0.01) pair-wise differences were detected.

Abbreviations: ADMA, asymmetric dimethylarginine; ARG, arginine; MDA, malondialdehyde.
Figure 4. Superficial femoral artery responses in the EX and REST trials. Data are means ± SE, n = 11. *P<0.05 from baseline. #P<0.05 between trials. Analyses were performed using 2-way RM ANOVA to determine main and interactive effects. Bonferroni’s post-test was used to evaluate pair-wise differences.

Time: P = 0.005  
Trial: P = 0.003  
Time x Trial: P = 0.545
Figure 5. Superficial femoral artery resting shear rate in the EX and REST trials. Data are means ± SE, n = 11. *P<0.05 from baseline. #P<0.05 between trials. Analyses were performed using 2-way RM ANOVA to determine main and interactive effects. Bonferroni’s post-test was used to evaluate pair-wise differences.
References


Consent Form for Participation in a Research Study

Principal Investigator: Kevin Ballard, Ph.D.

Study Title: Prior Aerobic Exercise to Attenuate Vascular Dysfunction Induced by Prolonged Sitting in Healthy Men

You are invited to participate in a research study that is designed to examine whether exercise helps to protect against events occurring with prolonged sitting that would impair blood vessel function (vascular function). You must be 18 years of age or older to participate in this study.

This consent form will give you the information you will need to understand why this study is being done and why you are being invited to participate. It will also describe what you will need to do to participate and any known risks, inconveniences or discomforts that you may have while participating. We encourage you to take some time to think this over and to discuss it with your family, friends and doctor. We also encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form.

PURPOSE OF THE STUDY

The purpose of this research study is to determine whether the performance of a single bout of treadmill exercise protects against decreases in vascular function that occur with prolonged sitting. By completing this study, we will have a more complete understanding of how exercise may protect vascular function during sedentary behavior.

RESEARCH PROCEDURES

This study will last approximately 2-4 weeks depending on your availability. If you would like to participate, we will perform the following tests and procedures:

Screening Visit (estimated time: 90-120 minutes):

Health History and Physical Activity Questionnaire: You will be asked to visit our study center after an overnight fast (>8 hours). For the 2 days prior to this visit, we will ask that you refrain from performing strenuous activity or exercise. We will ask that you complete a brief health history and physical activity questionnaire. This questionnaire will be similar to many of the forms you have likely completed at your doctor’s office. We’ll ask you to complete this form to ensure that it is safe for you to conduct the exercise tests, and because certain diseases or conditions could interfere with our ability to understand your data. Additionally, this questionnaire will allow us to determine how physically active you are. The questions will be related to what types of exercise (if any) in which you regularly participate. This questionnaire will take 10-15 minutes to complete.
Dietary Instruction: You will be instructed by study personnel to complete a 1 day food record leading into Trial 1. At Trial 1, you will receive a copy of your food record and asked to replicate your food record 1 day before Trial 2.

Height, Weight, Waist Circumference, and Body Composition Testing: In addition to measuring your height and weight, we will determine your waist circumference using a flexible tape measure. Your body composition will be measured by a technique that sends a small electric current through your body. These tests will take 10-15 minutes to complete.

Heart Rate, and Blood Pressure: After a 5 minute period of seated rest, heart rate and blood pressure will be measured. These tests will take 5-10 minutes to complete.

Blood Sampling: After a 10 minute period of seated rest, your blood will be drawn into tubes from an arm vein using sterile procedures. We will collect approximately two 10 milliliter tubes of blood. This amount is equal to approximately 1.5 tablespoons of blood. Briefly, the technician (Dr. Ballard or a phlebotomist) will clean the collection site with an alcohol wipe, a tourniquet will be applied to your upper arm, and a small needle will be inserted into an arm vein. Once the tubes are filled, the needle and tourniquet will be removed and pressure will be applied until any bleeding has stopped. The collection site will be covered with a sterile band-aid. We will try no more than 3 times to collect a blood sample. This procedure will take approximately 5 to 10 minutes. Your blood sample will be used to measure your levels of cholesterol, triglycerides, and glucose. You will be provided with a light snack (i.e., granola bar) following blood sampling.

Maximal Treadmill Test: You will also be asked to complete a maximal exercise test on a treadmill to determine you current cardiovascular fitness. This test will involve running on a treadmill at an increasing speed and incline until you are too tired to continue, and will last approximately 15 minutes. During this test, you will be equipped with a mouthpiece that will connect to a computer that is used to evaluate the air you breathe out as you run. This information will be used to determine the intensity that you are going to run at for the exercise intervention later in the study. Once the test is stopped, the speed and incline on the treadmill will be decreased and you will continue to walk slowly to cool down for 5 minutes. This test will take approximately 20 to 30 minutes to complete.

Trials 1 and 2 (estimated time: 6 hours per trial):

General Study Procedures: You will be scheduled at your convenience to visit our study center on two days separated by at least one week. You must not engage in any exercise for 2 days prior to each of the study test days. For each visit to our study center, you will need to be fasted for >8 hours (no food or drink, only water) to ensure reliable blood values.

This study is designed to take place as two trials (Trial 1 and Trial 2). Although the procedures for the trials are listed in order below, you will complete each of these trials in random order so that bias in the results of this study can be prevented.

During each trial of our study, we will be collecting 100 ml (7 tablespoons) of blood. Throughout the span of the study which consists of a screening blood sample and 2 study trials, we will be
collecting a total of 15 tablespoons (~1 cup) of blood. This amount of blood is less than the amount of blood that the Red Cross collects if you were to donate blood. This amount of blood should not affect you in any way, but you should not donate any blood during the course of this study.

**Blood Sampling:** After a 10 minute period of seated rest, your blood will be drawn into tubes from an arm vein using sterile procedures (described above). We will collect approximately two 10 milliliter tubes of blood at each time point.

**Ultrasound Test:** We will ask that you sit quietly for 10-15 minutes in our study center’s chair. We will then use a safe and noninvasive ultrasound monitor to locate the blood vessel in your right leg (groin) in order to monitor the dilation and contraction of your blood vessel. The ultrasound procedure will then be initiated by obtaining resting images (1 minute) of your leg’s blood vessel. We will then inflate a cuff around your lower right thigh leg to briefly (5 minutes) restrict blood flow and then we will rapidly deflate it so that we can obtain ultrasound images and blood flow measurements from your blood vessel for 3 minutes. This test will take approximately 15 to 20 minutes to complete. You will be provided with a light snack (i.e., granola bar) following this test.

**Treadmill Test or Seated Rest:** Next, you will be asked to perform 45 minutes of moderate-intensity treadmill exercise or 45 minutes of seated rest. Following exercise (or seated rest), you will be provided with a light snack (i.e., granola bar) identical to the one consumed before exercise.

**Prolonged Sitting Test:** One hour following exercise (or seated rest), vascular function will be reassessed and blood collected. Next, you will be asked to sit for 3 hours in a comfortable chair with back support without moving your legs or feet. You will be allowed to move your arms to use a computer or do reading during this test. Vascular function will be measured and blood collected at 1 hour intervals during this test. You can drink water, but you cannot eat any food. After you complete this test, you will be provided with a light snack (i.e., granola bar). This test will take 3 hours to complete.

**POTENTIAL RISKS**

The primary inconvenience for you is that you must visit our study center on two different days separated by approximately 1 week period. On these days, you will need to remain at our study center for approximately 6 hours to allow sufficient time for us to complete the testing and collection of blood samples. The total time commitment for this study is expected to be approximately 14 hours. Whenever possible, we will accommodate your schedule.

**Blood Collection.** During the blood drawing aspect of this investigation, only experienced individuals will be responsible for collecting blood samples. All blood drawing materials will be sterile and sanitary techniques will be used. You may experience a small initial pain attributed to inserting the needle and bruising may occasionally occur after the procedures are completed. In addition, you may experience lightheadedness or faintness which is common when people donate blood.
Ultrasound Measurements. Measurement of blood vessel dilation involves having a blood pressure cuff placed on your right leg. It will be periodically inflated to reduce blood flow for 5 minutes in order to cause your blood vessel to dilate. The cuff will then be rapidly deflated allowing the blood vessel to relax. You may feel a slight pain or tingling in your toes or leg which may be very uncomfortable for you during this brief period, but this sensation will go away shortly after the blood pressure cuff is deflated. The inflatable cuffs and procedures are similar to those used at your doctor’s office when you have your blood pressure checked.

Exercise Testing: Exercise and exercise testing can cause a heart attack or sudden death. This rarely happens: 1 in 10,000 tests in those with heart disease and at a much lower rate for healthy individuals. These events will be minimized by carefully watching how your body responds to exercise and your symptoms during exercise testing. Your exercise test will be performed by research staff that are CPR and AED (automated external defibrillator) trained. We have an AED located near the lab in Phillips Hall. An additional minor risk is muscle soreness for 1-3 days following the exercise test. This can be controlled by the use of over-the-counter medications such as aspirin.

POTENTIAL BENEFITS
While you may not directly benefit from participation in this study, you will be provided with information on your height, weight, waist circumference, and cardiovascular fitness. In addition, you will benefit indirectly from participating in this study by gaining insight into the role that exercise alters the way your blood vessels function. Overall, the results obtained from this study are expected to enhance our understanding of how exercise may protect vascular function during sedentary behavior. This knowledge is of great importance because it may potentially lead to additional health advice to reduce the risk of developing heart disease.

PROCEDURES NOT RELATED TO THIS RESEARCH
Blood samples collected for this study will be analyzed for cholesterol, glucose, triglyceride, oxidative stress markers, and markers associated with vascular function. Any remaining blood samples will be stored up to 5 years at our study center. These stored samples may be used for future additional blood marker measurements only if you allow it.

Do you agree to allow us to store any remaining blood samples for additional future measurements? Please circle your response and provide your initials below:

YES  NO  ______ (Participant’s Initials)

CONFIDENTIALITY
All of your data will be treated as confidential. Only Dr. Ballard and members of the research team will have access to personal information obtained on questionnaires or during testing. All of your data will be kept in a locked file cabinet in Dr. Ballard’s office, and any electronic data
generated will not be associated with your name. Upon enrollment, we will assign you a subject code that will allow us to identify samples and data without using your name.

**VOLUNTARY PARTICIPATION**

Your participation in this study is strictly voluntary. You are free to withdraw from participation for any reason, at any time without penalty or loss of benefits/compensation. You are also free to refuse to participate in any portion of this research study. Please be advised that this may result in the investigator discontinuing your participation in the remainder of the study. If you withdraw or are dismissed from our study, you will be paid for the completed aspects as indicated below.

**ALTERNATIVES**

An alternative would be to not participate in this study. Not participating in this study will not adversely affect you.

**REIMBURSEMENT FOR EXPENSES**

There will be no cost to you for participating in our study. If needed, we will give you a temporary parking passes to use for parking on the Miami campus.

You will be compensated for your time and travel. If you complete the screening visit but withdraw or are dismissed from our study prior to Trial 1, you will not be compensated. If you complete our study in its entirety, you will receive $120 at the completion of the study. For each of the two trials associated with this the study, you will be paid in the following manner:

- $60 will be provided for visiting our study center and completing Trial 1 in its entirety (6 hours).
- Therefore, if you complete the two trials of this study, you will be paid a total of $120 as outlined below.
  - $120 = $60 * 2 trials (6 hours per trial)
- If you begin a trial but are unable to complete the trial in its entirety, you will be paid for your time completed during that trial at a rate of $10 per hour ($60/6 hours = $10 per hour). For example, if you complete 3 hours of a trial but are unable to complete the remaining 3 hours you will be paid $30 ($10 x 3 hours). Reasons for non-completion of a study trial may include illness, fainting, or unwillingness to sit for 3 hours. If you are unable to complete Trial 1 in its entirety, you will be rescheduled for another date or disqualified from further participation in the study.

If you withdraw or are dismissed from our study, you will be paid for the completed aspects as indicated above. Reasons for study dismissal may include non-compliance to study procedures such as performing exercise during the 2 days preceding each trial or unwillingness to arrive at our study center in the fasted state. If you are dismissed from our study, Dr. Ballard will notify you immediately and you will be paid for the completed aspects as indicated above.

**COMPENSATION FOR INJURY**
In the event you are injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If you require medical care for such injury, your care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed. You will not receive compensation for medical bills.

**CONTACT INFORMATION**

If you have any complaints, concerns, input or questions regarding your rights as a subject participating in this study you may contact the Office for the Advancement of Research and Scholarship at (513) 529-3600 or humansubjects@miamioh.edu. If you have additional questions regarding the specifics of the study, please contact Dr. Kevin Ballard at (513) 529-9247 or ballarkd@miamioh.edu.

**SIGNATURES**

Informed consent is required of all participants in this research study. Whether or not you provide informed consent for this research study will have no effect on your current or future relationship with Miami University. By signing below, you acknowledge that the purpose, procedures, and risks/benefits of participation in this study have been described to you, and that any questions you may have had were adequately addressed by a member of Dr. Ballard’s research team.

____________________  ______________________  _________
Participant Signature  Print Name  Date

____________________  ______________________  _________
Signature of Person Obtaining Consent  Print Name  Date
APPENDIX B

PHONE SCRIPT

Thank you for calling about our study. ANY INFORMATION GIVEN IN THIS INTERVIEW WILL BE KEPT CONFIDENTIAL.

The purpose of this study is to evaluate the short term effects of a single bout of treadmill exercise on blood vessel responses to prolonged sitting. For this study, we are looking to recruit men between the ages of 18-30 who are currently physically inactive.

CAN I ASK YOU SOME QUESTIONS?

1. You will need to come to the study center for about 6 hours on two separate days separated by at least 1 week. During each visit, we will be collecting blood samples and using a noninvasive ultrasound technique to monitor your blood vessel’s ability to constrict and dilate. The two phases of the study will be completed in a random order. During one visit, we will ask you to run/walk on a treadmill for 45 minutes and then we will collect blood samples and assess the health of your blood vessel. During another visit, we will ask you to sit quietly for 45 minutes and then we will collect blood samples and assess your blood vessel function. Is this something that still interests you and will it fit your schedule?

   YES  NO

   If “No”, the caller will be thanked for his interest.

2. Are you between the ages of 18-30?  

   YES  NO

   If “No”, the caller will be thanked for his interest and told that we only recruiting individuals of this age range.

3. Are you female?  

   YES  NO

   If “Yes”, the caller will be thanked for his interest and told that we only recruiting males.

4. Do you take vitamins?  

   YES  NO

5. Do you take vitamin E?  

   YES  NO
6. How about vitamin C? YES NO

7. Do you take any antioxidant supplements or fish oils? YES NO

If “Yes” to any of these questions, then the caller will be thanked for their interest and told that we need subjects who do not take supplements.

8. Do you use tobacco products? YES NO

If “Yes”, then the caller will be thanked for their interest and told that we need subjects who do not use tobacco products.

9. Do you consume more than 3 alcoholic beverages per day or more than 10 alcoholic beverages per week? YES NO

If “Yes”, then the caller will be thanked for their interest and told that we need subjects who do not use regularly drink alcoholic beverages.

10. Do you exercise on a regular basis? YES NO

If “Yes”, how often and what activities?

________________________________________________________
________________________________________________________
________________________________________________________
________________________________________________________

11. Do you have any lower extremity injuries that would prevent you from exercising vigorously on a treadmill? YES NO

If “Yes”, please describe:

________________________________________________________
________________________________________________________
________________________________________________________
________________________________________________________

12. We are specifically recruiting individuals who are not overweight. Do you consider yourself as overweight? YES NO
What are your height and weight?

________________________________

13. We are now going to read a number of disorders and health conditions to you. If you have any of these conditions, you will not be able to participate in our study. Please be advised that we do not want you to answer “yes” or “no” to any of these conditions.

- HIV, hepatitis, or bleeding disorders such as hemophilia
- Use of statins or any drugs to control your blood lipids such as cholesterol or triglycerides
- Hypertension/high blood pressure or taking any drugs to control blood pressure
- Any drugs to control diabetes
- Any history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease

Do you still want to participate in our study?  

YES  NO

If “No”, the caller will be thanked for their interest in our study

If “Yes”, the interview/screening will continue as indicated below

14. Have you ever had your blood drawn?  

YES  NO

15. If “Yes”, have you ever felt faint, light headed or lost consciousness after getting your blood drawn?  

YES  NO

16. Would you be willing to come to a screening to have a blood sample taken?  

YES  NO

If “Yes”, the subject would be told a date to come to the screening and to arrive fasting. They then would be told that the following procedures will be carried out:

**PROCEDURES**

**Screening Visit.**
You will be asked to come to a pre-study screening visit to learn about our study and to provide a fasting blood sample. If you have eaten or consumed anything other than water for the past 8 hours, you are not eligible to give a screening blood sample at this time. We will describe our study, give you a consent form to read, provide you time to ask any questions, and then have you sign the consent form if you choose to participate. Then we will measure your blood pressure, your height, weight, and waist circumference, and a blood sample will be drawn to measure your blood chemistries such as cholesterol and glucose. Next, you will also be asked to complete a maximal exercise test on a treadmill to determine your current cardiovascular fitness. This test will involve running on a treadmill at an increasing speed and incline until you are too tired to continue, and will last approximately 15 minutes. During this test, you will be equipped with a mouthpiece that will connect to a computer that is used to evaluate the air you breathe out as you run. We expect that you should be able to begin our study within 1-2 weeks following the pre-screening meeting.

What participants will do during the study?

If you decide to volunteer, we will ask you to come fasted to our study center on two different days. After your first visit, you will need to visit our center again after about a week. For 48 hours prior to you visiting our study center, we ask that you do not exercise as it will greatly alter our study’s results. In addition, we will ask that you record all foods and beverages consumed for 24 hours prior to each visit.

On each day that you come to our study center, we will collect a blood sample from your arm. During each visit, we will collect a total of 100 ml (7 tablespoons) of blood or 15 tablespoons (~1 cup) throughout the entire study. In addition, during each visit, we will be using an ultrasound machine to monitor the way a blood vessel in your leg dilates and contracts and to evaluate the health of your artery as it relates to the risk for heart disease. You will be provided with a light snack following this test.

These procedures will be conducted identically on two separate occasions with one exception. During one visit, you will run on a treadmill for 45 minutes. In another visit, you will sit quietly for 45 minutes. You will be provided with a light snack following exercise or seated rest. One hour following exercise (or seated rest), vascular function will be re-assessed and blood collected. Next, you will be asked to sit for 3 hours in a comfortable chair with back support without moving your legs or feet. You will be allowed to move your arms to use a computer or do reading during this test. Vascular function will be measured and blood collected at 1 hour intervals during this test. You can drink water, but you cannot eat any food.

**ARE YOU INTERESTED IN PARTICIPATING IN THE STUDY?**

**YES**  **NO**

If “No”, the caller will be thanked for their interest

If “Yes”, the caller will be asked for the following information
• Could we have your name and day and evening phone numbers as well as your email address?

• Potential participants will be given information as to how to come to our study center for the pre-study screening.
APPENDIX C

Medical History and Physical Activity Questionnaire

Subject Initials: ______
Subject ID: ______
Collected by (initials): ______

Demographics

Date of Birth: ___/___/____
Age (years): ______
Race: 
   _____ Caucasian
   _____ Native Hawaiian or Pacific Islander
   _____ African American
   _____ American Indian or Alaskan Native
   _____ Asian
   _____ Other (specify)

Do you currently use tobacco products? _____ Yes _____ No

On average, how many alcoholic drinks do you consume daily? ______
On average, how many alcoholic drinks do you consume per week? ______

Medical History

Please list any allergies, injuries, or pertinent health conditions.
_____ None

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<th>Former and/or Present Conditions</th>
<th>Approximate Date</th>
<th>Condition still present? (Y/N)</th>
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</table>


Medications

Please list all medications and dietary supplements you are currently taking.
_____ None

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<th>Frequency</th>
<th>Start Date</th>
<th>Stop Date</th>
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Physical Activity

On average, how many days per week do you perform physical activity that is either moderate intensity (moderately out of breath) or vigorous intensity (breathing hard)?
_____ days per week

For how long have you been doing these types of activities?
_____ months/years

How many minutes does a typical session of activity last?
_____ minutes

Additional Comments
____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
APPENDIX D

Name: ________________________

1 Day Food Diary

Directions for using the Food Diary

1. Please record all foods and beverages (including water) consumed for each day.
2. The food diary will be easier to use if you record the foods immediately after they are consumed. Make sure to include all foods eaten.
3. Record only one food item per line.
4. **Please try to be as specific as possible** when describing how the food item was eaten or prepared—for example: fresh, frozen, fried, baked, canned, boiled, or raw.
5. Include **brand names** when possible. You may include empty packages or wrappings of consumed food items to help us identify the food.
6. Include attachments of recipes of homemade food whenever applicable.
7. When recording amounts please try to use **household measures**—for example: ounces, tablespoons, cups or units such as two slices of toast, or one raw apple.
8. For canned foods, please include what liquid the food item is in—for example: tuna in water.
9. Please **try not to alter from your normal diet** during the recording period.
10. Also remember to record the fats and oils that you eat or use for cooking—for example: salad dressing, oils, butter, margarine, etc.

* The following are examples of some of the ways to list food items and amounts.

<table>
<thead>
<tr>
<th>Time</th>
<th>Food Item and Method of Preparation</th>
<th>Amount Eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>8am</td>
<td>Sliced white bread, toasted, homemade (see recipe attached – bread was sliced into 16 slices)</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Coffee</td>
<td>10 oz</td>
</tr>
<tr>
<td></td>
<td>Maxwell House Ground Coffee Master Blend (1/4 C ground coffee with 3 C water)</td>
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</tr>
<tr>
<td></td>
<td>White granulated sugar</td>
<td>1 tsp</td>
</tr>
<tr>
<td></td>
<td>Milk, skim, Stop &amp; Shop brand</td>
<td>1 oz</td>
</tr>
<tr>
<td>Time</td>
<td>Meal</td>
<td>Ingredients</td>
</tr>
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<td>-------</td>
<td>-------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>12:30</td>
<td>Cheeseburger, grilled</td>
<td>1 95% lean beef patty, ¼ pound, Cheese, Kraft Singles American cheese, 1 slice, Hamburger buns, Pepperidge Farms Classic Hamburger buns, 1 Ketchup, Heinz, 1 Tbsp, Yellow mustard, Stop &amp; Shop brand, 1 Tbsp, Coca Cola, Classic, 1 can (12 oz), French fries, Ore-Ida Golden Crinkles, frozen french fries, baked at 400˚C for 20 mins, 2 cups, cooked</td>
</tr>
<tr>
<td>2pm</td>
<td>Sour cream and onion potato chips, Lay’s</td>
<td>2 oz bag, Water, 16 oz</td>
</tr>
</tbody>
</table>
One serving size is:

- ¾ cup (6 ounces) 100 percent fruit or vegetable juice
- 1 medium piece of fruit (apple, orange, banana, pear)
- ½ cup raw, cooked, frozen, or canned fruit (melon, fruit cocktail, applesauce)
- ½ cup raw, cooked, frozen or canned vegetables
- 1 cup raw leafy vegetables (salad greens)
- ¼ cup dried fruit (raisins, plums/prunes, apricots)
- ½ cup cooked, canned or frozen peas and beans (legumes)

What does that look like on my plate?

- 3 ounces of chicken, meat, or fish is the thickness and size of a deck of cards
- 1 oz of cheese is 1 thumb, 3 dominoes, or 1 small hotel soap
- ½ cup of a fruit, vegetable or legume looks like a scoop of ice cream
- 1 cup of salad is the size of a fist or baseball
- ¼ cup of dried fruit would fit in a golf ball
- 1 medium fruit is the size of a tennis ball
- 1 oz of snack food is the size of a handful of snack food
- 1 can of soda is 12 ounce; a small bottle is 20 ounces
- ½ tsp is the size of the tip of your index finger (to first joint line)
- 1 tsp is the size of the tip of your thumb (to first joint line)
- 1 Tbsp is the size of 3 thumb tips
VEGETABLE SERVING SIZES

Avocado (Half)  Mushrooms (14 pieces)

Baby sweetcorn (6 pieces)  Parsnip (1 large)

Brussels sprouts (8 pieces)  Peas, fresh, frozen or canned
                                (3 heaped tbsp or 1/2 cup)

Cabbage  Scallions (8 pieces)

      (3 heaped tbsp shredded or 1/2 cup)

Carrots (1 large)  Snowpeas (Handful)

Chickpeas (1/2 cup)  Sugarsnap Peas (Handful)

Mixed salad (1 cup)  Vegetable soup

      (1 serving of fresh or canned soup)

Kidney beans (1/2 cup)  Zucchini (Half a large one)

FRUIT SERVING SIZES

Apricots, dried (3 whole)  Pear (1 medium)

Banana (1 medium)  Pineapple, canned (2 rings)

Figs, fresh (2)  Pineapple, fresh (1 large slice)

Mango (2 slices)  Plums (2 medium)

Melon (1 large slice)  Peach or Nectarine (1 medium)
**EXAMPLES OF 1/2 CUP**

<table>
<thead>
<tr>
<th>1 snack container of applesauce (4oz)</th>
<th>16 grapes</th>
<th>1 medium cantaloupe wedge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2 medium grapefruit</td>
<td>4 large strawberries</td>
<td>5 broccoli florets</td>
</tr>
<tr>
<td>6 baby carrots</td>
<td>1 large plum</td>
<td>1 small box (1/4 cup) of raisins</td>
</tr>
</tbody>
</table>
EXAMPLES OF 1 CUP

1 small apple
1 large banana
1 medium grapefruit

2 large or 3 medium plums
8 large strawberries
1 large bell pepper

1 medium potato
2 large stalks of celery
1 cup cooked greens or 2 cups raw (spinach, collards, mustard greens, turnip greens)
12 baby carrots
(or 2 medium carrots)

1 large sweet potato

1 large ear of corn
APPENDIX E

Maximal Treadmill Exercise Test
Instructions: Make sure that the subject is ready to begin, and that you have explained the protocol to them. Make sure that the mouthpiece is properly assembled, the VO2 cart is calibrated, and that you are ready to begin the test. Consult the checklist to ensure that you haven’t missed anything.

Maximal Treadmill Test

<table>
<thead>
<tr>
<th>Stage</th>
<th>Speed (mph)</th>
<th>Grade (%)</th>
<th>Stage Duration (min)</th>
<th>HR (bpm)</th>
<th>RPE</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm-Up</td>
<td>3.0</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.0</td>
<td>5.0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>7.5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>10.0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>12.5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>15</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cool Down</td>
<td>3.0</td>
<td>0</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments____________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________

VO\textsubscript{2}max: __________ ml/kg/min __________ L/min

HR\textsubscript{max}: __________ bpm Final RPE: __________ RER @ max: __________
Date & Time of visit 1: ________________________________
Robert M. Duguid

EDUCATION

- August 2011 – May 2015
  Grove City College
  - Bachelor of Science in Exercise Science
  - Minors in Biology and Psychology
  - GPA: 3.82/4.00 – 8 semesters
  - Major GPA: 3.84/4.00 – 8 semesters

- August 2015 – May 2017
  Miami University
  - Masters of Kinesiology
  - Graduate GPA: 3.78/4.00 – 4 semesters
  - Undergraduate GPA: 3.94/4.00 – 1 semester

PROFESSIONAL EXPERIENCE

- August 2015 – May 2017
  Miami University
  Graduate Teaching Assistant
  - Teach laboratory classes and assist faculty with ongoing research and lecture work

- May 2014 – May 2015
  Grove City YMCA
  Fitness Specialist
  - Assist with personal training, instructing group exercise, and managing the operations of the fitness center

- January 2012 – May 2015
  Grove City College
  Cellular and Molecular Biology TA
  - Help to run the lab experiments, grade lab reports, and assist students with questions during and after lab hours

- May 2013 – May 2015
  Grove City College Technological Learning Center
  Helpdesk Employee
  - Aid students, faculty, and staff with hardware and software related computer problems

EXTRACURRICULAR LEADERSHIP EXPERIENCE
May 2010 – Present
Grove City YMCA, Grove City College, Miami University
Zumba® Fitness Instructor
  • Led students in a Latin-inspired dance workout that mixes the fun of a dance party with the intensity of an aerobics class

RESEARCH EXPERIENCE AND INTERESTS
Previous Studies:
- Effects of Kinesio Tape on biceps brachii strength
- Functional Movement Screen testing on female college-aged lacrosse players before and after season
- Hamstring/Quadriceps strength ratios and force plate balance analysis of college athletes
- Effects of closed and open kinetic chain training interventions on markers of patellofemoral pain
- Effects of aerobic exercise on vascular function during prolonged sitting in healthy men
- Effects of endurance and resistance training on vascular responses to glucose ingestion

Fields of Interest:
- Clinical exercise physiology prescription and outcomes
- Medical education and outcomes
- Health policy and preventable diseases

ACTIVITIES
- Grove City College Exercise Science Club
  Chaplain, August 2013 – May 2015
  • Helped to establish the club and draft the constitution, as well as establishing events such as the spring 5k, fundraising Zumba® event, and Exercise Science Symposium.
- Miami University Kinesiology Graduate Committee
  Graduate Student Liaison, August 2015 – May 2017
  • Consulted the graduate committee on academic and policy matters concerning the graduate students and graduate assistants

PROFESSIONAL MEMBERSHIPS AND AWARDS
- American College of Sports Medicine, June 2014 – Present
- National Strength and Conditioning Association, October 2014 – Present
  o Certified Strength and Conditioning Specialist – December 2014 - Present
- American Heart Association – CPR/AED, October 2012 – October 2014
- American Red Cross – CPR/AED, October 2014 – Present
- President’s Scholarship – Fall 2012 – May 2015
- Dean’s List – Spring 2012 – May 2015

HOBBIES AND EXTRACURRICULAR INTERESTS

- Massage Therapy, Exercise and Health Community Education, Social Dancing