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Arterial adaptations to training among first time marathoners

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Abstract

Increased arterial wall thickness has been positively related to cardiovascular disease. Exercise has been found to favorably alter arterial anatomy in the trained limbs, but its effects on untrained limbs and the carotid artery are unclear. Thus, brachial (non-trained limb), popliteal (trained limb) and carotid, wall thickness (WT), wall to lumen diameter ratios (W:L), intima-media thickness (IMT) and lumen diameters (LD) were compared between recreationally active (n=14) and untrained (n=11) subjects before and after, the active subjects participated in 12 weeks of marathon run training. Arterial dimensions were measured with B-mode ultrasonography. Pre and post-training VO_{2max} and running speed at 3.5mmol lactate were measured in the marathon training group; VO_{2max} was unaltered, but running speed increased from 7.8 ± 0.7mph to 8.2 ± 0.9 mph (p=.008). Time by group interactions were observed for the brachial and popliteal measures ($p<0.05$), but not in the carotid, and no differences were observed in the control group. Prior to the intervention the marathon training group had significantly larger LD in the brachial (p=.002) and popliteal arteries (p=.007) than the control group; no other pre-training differences were found. Following training, WT was reduced in the brachial (pre = .99 ± .16mm; post = .84 ± .10mm; p=.007) and popliteal (pre = .96 ± .09mm; post = .86 ± .11mm; p=.005) arteries, and was characterized, in part, by a 0.07mm decrease in brachial IMT (p=.032) and a non-significant 0.03mm reduction in popliteal IMT. LD increased in the brachial (pre = 3.38 ± .35mm; post = 3.57 ± .41mm; p=.015) and popliteal (pre = 4.73 ± .48mm; post = 5.11 ± .72mm; p=.002) arteries. These data suggest that arteries in the trained and non-trained limbs are favorably altered by regular exercise and that the alterations may be exercise dose dependent.

Chapter I

Introduction

Exercise provides a potent physiological stimulus that reduces cardiovascular disease risk (Paffenbarger et al., 1986). The cardioprotective effects of exercise may be partially explained by arterial remodeling that favors improved compliance and increased blood flow (Joyner and Green, 2009) as well as vessel wall adaptations that are inversely associated with hypertension (Folkow, Grimby and Thulesius, 1958), atherosclerotic disease (Lorenz et al., 2007), myocardial infarction and stroke (O'Leary et al., 1999). More specifically, exercise training has been found to improve vascular function by decreasing arterial wall thickness (WT), wall-to-lumen diameter ratios (W:L), intima-media thickness (IMT) and increasing lumen diameter (LD) (O'Leary et al., 1999; Dinenno et al., 2001; Moreau et al., 2006; Thijssen et al., 2007; Thijssen et al., 2011). What remains unclear is whether these adaptations are localized to the trained limbs or whether they are also manifested in the arteries of non-trained limbs as well as the carotid arteries.

Exercise and Arterial Dimensions

Trained Limbs A variety of research shows that within the trained limbs, regular exercise is associated with lower vessel WT, IMT and W:L and greater LD. Cross-sectional data reported by Dinenno et al. (2001) indicates that endurance trained runners and triathletes have larger femoral artery LD and smaller femoral IMT and IMT-to-LD ratio when compared to sedentary control subjects. Similarly, Moreau et al. (2006) reported that femoral IMT was lower in endurance trained men and women regardless of

age; and, Schmidt-Trucksass et al. (2000) found that femoral LD was larger in runners and cyclist when compared to sedentary controls and subjects with spinal cord injuries. Limiting the subject pool to those that participated in upper body exercises, Rowley et al. (2011) showed that brachial LD was greater and WT and W:L were smaller in canoeist when compared to sedentary subjects. In a separate study, these same researchers (Rowley et al., 2011) found that brachial LD was greater, but that vessel WT was no

Table 1. Cross-sectional studies investigating the impact of physical activity on vascular measures

The 'Effect' indicates that a higher physical activity level is related lower arterial measures (-), higher arterial measures (+) or no difference in arterial measures (~). Exercise history relates to the comparison between the groups that differ in training history. CA, carotid artery; BA, brachial artery; FA, femoral artery.

First author	*n*	Age (years)	Fitness measure	Artery	Effect (CA, BA, FA)			
					IMT	WT	W:L	LD
Dinenno	108		Exercise test	BA, FA	N/A -			~ +
Moreau	173	20-79	Exercise test	FA	-			
Schmidt-Trucksass	51		Exercise test	CA, FA	~ ~			~ +
Rowley	38			CA, BA, FA		- - -	- - -	~ + +
Rowley	29			CA, BA, FA		- - -	- - -	N/A + +
Green	31		Exercise test	BA, PA		- -	- -	~ +
Gando	771	40-60	Exercise test	CA	-			-

different, in the dominant arm compared to the non-dominant arm in competitive squash players. Experimental evidence further suggests that arterial WT (Green et al., 2010;

Thijssen et al., 2011), IMT (Dinenno et al., 2001; Spence et al., 2012) and W:L (Green et al., 2010; Thijssen et al., 2011) decrease with training while LD increases (Dinenno et al., 2001; Thijssen et al., 2007; Green et al., 2010, Spence et al., 2012). The only contradictory data found among the research was presented by Thijssen et al. (2007) who reported that, while common femoral artery LD increased, superficial femoral LD did not change in older adults following eight weeks of leg ergometer training. Limitations in this study may have been the relatively short time course of the training program and inadequate statistical power associated with a low sample size of eight subjects (Thijssen et al., 2007).

Non-trained Limbs When compared to trained limbs, regular exercise appears to have a limited effect on the arteries in the non-trained limbs (e.g., arms during cycling or running). In general, the available cross-sectional and longitudinal research suggest that brachial artery WT (Green et al., 2010; Rowley et al., 2011; Rowley et al., 2011) and W:L (Green et al., 2010; Rowley et al., 2011) is lower among trained subjects, but unlike the adaptations found in the trained limbs, IMT (Thijssen et al., 2007) and LD (Dinenno et al., 2001; Green et al., 2010; Thijssen et al., 2011; Spence et al., 2012) are not different. In contrast to the data reported by others, Maiorana et al. (2011) found that WT and W:L in the brachial artery decreased in chronic heart failure subjects participating in twelve weeks of lower body resistance training, but not those participating in lower body aerobic training. These researchers (Maiorana et al., 2011) also showed that brachial LD increased in patients from both groups. Comparison of the results from Maiorana et al. (2011) to the other studies is difficult because the subjects in this study were patients with heart disease and those in the other studies reviewed were identified as healthy adults.

Carotid Artery The relationship between carotid artery dimensions, especially carotid IMT, and exercise has been widely studied. In a recent review, Thijssen, Cable and Green (2012) presented a variety of cross-sectional data supporting the position that exercise is associated with lower carotid artery IMT. Among the 22 studies reviewed, 18 suggested that carotid IMT was lower in subjects that were either more active or scored higher on fitness tests (e.g., maximal oxygen consumption). Experimental data reviewed by these authors was less convincing, however, with two of five studies showing that carotid IMT was favorably influenced by training. Specifically, in two multiyear studies in which the duration of the training ranged from 204 (Wildman et al., 2004) to 312 (Rauramma et al., 2004) weeks a progressive age-related increase in carotid IMT was attenuated, with training, but three studies involving relative short duration training programs (i.e., 8 to 12 weeks) did not show any differences.

Recent studies not included in the review by Thijssen, Cable and Green, (2012) provide limited additional insight. Whereas some of the remaining evidence suggests that carotid IMT (Schmidt-Trucksass et al., 2000) as well as LD (Rowley et al., 2011; Rowley et al., 2011; Schmidt-Trucksass et al., 2000; Spence et al., 2012) are not related to physical activity, there are notable exceptions (Gando et al., 2011; Spence et al., 2012). In a large scale (n = 771 adults), cross-sectional study Gando et al. (2011) found that age associated increases in carotid IMT and LD were attenuated in subjects with higher cardiorespiratory fitness; and, following a randomized, longitudinal study Spence et al. (2012) reported that 6 months of either endurance or resistance training lead to significant reductions in carotid IMT. Two additional studies (Rowley et al., 2011, 2011) were found

that evaluated the relationship between exercise and carotid artery WT and W:L. In both studies, WT and W:L was lower in athletes when compared to control subjects.

Table 2. Experimental studies investigating the impact of exercise training on vascular measures

The 'Effect' of the intervention relates to a decrease (-), increase (+), or no change (~) in arterial measures. CA, carotid artery; BA, brachial artery; CFA, common femoral artery; SFA, superficial femoral artery.

First author	n	Age (years)	Type of training	Weeks	Artery	Effect (CA, BA, FA)			
						IMT	WT	W:L	LD
Thijssen	11		Simultaneous handgrip exercise	8	BA		-	~	-
Thijssen	8	70±1	Cycle training	8	CA, BA, CFA, SFA	~ N/A N/A ~			~ ~ + N/A
Maiorana	36		Lower body resistance or aerobic training	12	BA		-	-	+
Rauramma	140		Aerobic training	312	CA	~			
Wildman	353	44-50	Lifestyle intervention	204	CA	~			
Spence	27		Endurance or resistance training	24	CA, BA, FA	- ~ ~		- - ~	N/A + +

Summary There is strong evidence indicating that physical activity and exercise training is associated with lower WT, W:L, IMT and larger LD in the trained limbs. The evidence related to non-trained limbs, while limited in scope, suggests that exercise may have a comparatively muted effect that results in lower WT and W:L, but little or no impact on IMT or LD. Data related to the carotid arteries is more equivocal with cross-

sectional research suggesting that physical activity and aerobic fitness are inversely correlated to IMT while the mixed results from experimental research fails to provide definitive support.

Limitations in the Existing Research

A notable weakness within the cited research is the scarcity of data from studies that simultaneously assess arterial WT, W:L, IMT and LD in trained limbs, non-trained limbs and the carotid artery. Not measuring arterial dimensions in all three regions limits the ability to determine whether a particular type or volume of exercise causes remodeling in the trained limbs only, or if changes are also manifested in arteries that are peripheral to the trained limbs. Among the studies reviewed, only Rowley et al. (2011, 2011) and Spence et al. (2012) simultaneously assessed the carotid artery and arteries within the trained and untrained limbs. As previously mentioned, these investigators (Rowley et al., 2011, Rowley, et al., 2011; Spence et al., 2012) found that LD was greater in the trained limbs but not in the carotid artery and the untrained limb. Rowley et al., also reported that arterial WT (2011, 2011) and W:L (2011) among trained subjects were lower in the trained limb, untrained limb and carotid artery as compared to controls; and, Spence et al. (2012) found that resistance and endurance training reduced carotid IMT. Since IMT is a component of WT, it is plausible that the carotid WT and IMT data is in agreement; however, due to a lack of information provided in the report it is unclear whether or not Spence et al. (2012) differentiated between WT and IMT in the brachial and femoral arteries. Given the limited data, it is evident that more research is needed to determine the simultaneous effects of exercise interventions on arteries from different

regions. Furthermore, this approach would make it possible to elucidate how different types and volumes of exercise training is related to local arterial remodeling as well as remodeling in arteries that are peripheral to the trained limbs.

Interaction between Exercise and the Mechanisms for Arterial Remodeling

A review of the mechanisms that interact with exercise may provide insight into whether or not arterial remodeling is likely to be limited to the trained arteries or if it is likely to be more widespread. Recently, Thijssen, Cable and Green (2012) divided the potential mechanisms that influence arterial anatomy, and function, into three categories. These include: 1) local hemodynamic stimuli (i.e., shear stress), 2) systemic hemodynamic stimuli (i.e., arterial pressure), and 3) systemic non-hemodynamic stimuli (i.e., sympathetic nervous system activity, inflammation and reactive oxygen species (ROS)). In their discussion, the authors briefly point out that higher levels of sympathetic nervous system activity are inversely associated with arterial WT and that elevated inflammation and a net accumulation of ROS are positively associated with IMT. They provide greater detail regarding the other mechanisms, suggesting that even though one is locally acting and the other systemic, repeated bouts of exercise alters shear stress and arterial pressure, which in turn alters nitric oxide (NO) synthesis in a way that favors increased LD and diminished WT. The author's attention to these mechanisms seems warranted given the evidence suggesting that they are primary mechanisms for arterial remodeling (Rudic et al., 1997; Maiorana et al., 2003; Newcomer et al., 2011).

Among the potential mechanisms effecting arterial remodeling, shear stress has been the most widely researched. Shear stress is a tractive force exerted at a vector

parallel to the long axis of the vessel and is characterized as an increase in blood flow that is proportionally greater than changes in vessel diameter (Niebauer and Cooke, 1996). When blood flow is increased, shear stress is imparted to the endothelium and induces ATP and substance P release from the endothelial cells of the vascular beds (Maiorana et al., 2003). Both ATP and substance P bind with endothelial receptors and stimulate the release of endothelial nitric oxide synthase (eNOS) (Shen et al., 1995). eNOS then converts the amino acid L-arginine into NO, which diffuses to the underlying vascular smooth muscle in the blood vessels to activate guanylate cyclase. Guanylate cyclase activates cyclic guanosine monophosphate and opens calcium channels, causing smooth muscle relaxation and vasodilation of the vessel.

In the early weeks of exercise training, the repeated increases in shear stress up-regulates the NO-dilator system, including eNOS, to buffer the increased shear (Maiorana et al., 2003). The up-regulation of the NO-dilator system also stimulates a variety of growth factors (e.g., endothelial derived growth factor). These growth factors modulate vascular remodeling (increased LD and decreased WT). With repeated, chronic exercise training, arterial remodeling ultimately normalizes the shear stress acting on the vessel and NO activity returns to pre-stimulus levels.

In contrast to chronic exercise and increases in shear, Rudic et al. (1998) showed that a decrease in blood flow and the absence of eNOS expression lead to a doubling in WT and reduced LD remodeling that was attributed to proliferation of vascular smooth muscle cells. In combination, this evidence suggest that periodic increases in shear, such as that seen with repeated bouts of exercise, attenuates arterial smooth muscle cell proliferation, thus leading to thinning of the arterial wall and expansion of the LD.

Similar to shear stress, transient increases in arterial pressure have been found to activate the NO mechanism; and, the cyclical increases in pressure that accompany exercise training are postulated to cause favorable changes in arterial structure (Maiorana et al., 2003). It is important to note that sustained higher pressures associated with hypertension, have deleterious effects on the arteries (Maiorana et al., 2003). Hypertension is characterized by endothelial dysfunction which is a result of attenuated NO expression. As a result of hypertension, ROS increases and and scavenges NO, thus decreasing NO bioavailability (Hermann et al., 2006). Reductions in NO resulting from hypertension are associated with the thickening of the arterial walls. In contrast, the cyclical changes in arterial pressure, associated with exercise augment NO bioactivity and thus favor reductions in WT and increases in LD (Maiorana et al, 2003).

Thijssen, Cable and Green, (2012) provide evidenced based support for the potential mechanisms identified above; and, categorizing them, as they did, as strictly "local" or "systemic" may simplify the discussion. Such categorizations, however, may also be misleading. For example, to state that inflammation is a "systemic" mechanism represents a limited perspective if one considers that muscle damage localized to the training limbs may have a different effect on vascular inflammation in proximity of the damaged tissue than on vessels more distal to the damage. Similarly, categorizing shear stress as a "locally" active mechanism may also be problematic. While shear forces adequate to stimulate arterial remodeling may indeed be restricted to local vessels when exercise intensity and/or duration is relatively low, during higher intensity, longer duration exercise, shear forces may have a greater peripheral effect.

A number of researchers (Ahlborg et al., 1975; Bangsbo et al., 1995; Green et al., 2002; Green et al., 2005; Tanaka et al., 2006) have at least partially addressed this issue by assessing blood flow in nonworking limbs during exercise. Tanaka et al. (2006) found that blood flow to the non-active limbs increased markedly in proportion to exercise intensity and that at peak exercise intensity, brachial blood flow during leg exercise was increased fourfold. These researchers (Tanaka et al., 2006) reported similar results in the femoral artery during arm exercise. Other studies using moderate arm ergometry exercise (Ahlborg et al., 1975; Bangsbo et al., 1995) or lower limb exercise (Green et al., 2002; Green et al., 2005) also showed that blood flow in the inactive limbs increased significantly. Blood flow to the carotid artery during exercise does not seem to be amplified as it is in the arms or legs, with Hellstrom et al. (1996) reporting that the maximum change in blood flow to the head during exercise is ~30-40% higher than at rest.

Even if the peripheral effect is slight, significant arterial remodeling, or, as reported by Rauramaa, et al. (2004), attenuation of wall thickening, may result over the course of months and even years of training. Hence it is plausible that discrepancies in the literature related to the effect of exercise training on arterial remodeling in non-trained limbs and the carotid artery may be at least partially due to differences in the training intensity and/or duration. And, while elucidating the mechanisms involved in arterial remodeling is important, it has been suggested that it is also important to better understand how exercise training intensity and duration is related to remodeling (Thijssen, Cable and Green, 2012; Spence et al., 2012). .

Rationale, Purpose and Hypotheses

The cardioprotective effect of exercise is associated with arterial remodeling that favors improved compliance and increased blood flow (Joyner and Green, 2009) as well as vessel wall adaptations that are inversely related to cardiovascular disease (Folkow, Grimby and Thulesius, 1958; Lorenz et al., 2007). Despite the potential widespread benefits, current research related to the effects of exercise on arterial remodeling is often limited to single artery assessment, usually involving either the trained limb or the carotid artery. Given that exercise may elicit local as well as peripheral arterial adaptations and that the extent of the adaptation may be related to exercise intensity and duration, research that simultaneously compares the effect of progressive exercise training on arterial dimensions in the trained and non-trained limbs as well as the carotid artery is warranted.

The present study was designed to compare WT, W:L, IMT and LD in trained, non-trained and carotid arteries between recreationally active and untrained subjects and to evaluate whether or not 12 weeks of marathon run training among the recreationally active subjects leads to arterial remodeling. The marathon training group was required to demonstrate the ability to continuously run a minimum of five miles at the beginning of the study and to subsequently engage in marathon run training for the remainder of the study. The age-matched controls were less active than the training group at the beginning of the study and maintained their activity levels throughout the training period. Comparisons were also made between and within the two groups before training.

It was hypothesized that due to differences in exercise behaviors, 1) the training group would have significantly lower WT, W:L and IMT, and greater LD than the

control group in the brachial and popliteal arteries, but not the carotid artery prior to participating in the marathon training program. It was also hypothesized that, 2) there would be a significant interaction between the groups due to the training as characterized by a) a further reduction in WT, W:L and IMT, and an increase in LD in the brachial and popliteal arteries in the training group and b) a reduction in the WT, W:L and IMT, and increase LD in the carotid artery in the training group.

Chapter II

Methods

Subjects

Fourteen male (n = 5) and female (n = 9) students from a university taught marathon running class and 11 inactive students volunteered to participate in this study. All methods for the study were approved by the James Madison University Institutional Review Board prior to commencement.

Eligibility requirements for the experimental group included the ability to complete a five mile continuous run prior to beginning the training program as well as having not previously run a marathon. After enrolling in the class, participants followed a 16-week marathon-training program. Pre and post-test data were collected before and after a 12-week segment within the training program. The program included four days of training per week and was divided into two phases. The first phase consisted of a 13-week training period that gradually increased the overall training volume by ~140% between week one and week 13. The peak running volume occurred in weeks 12 and 13 with a total of 36 miles run during each of those weeks. The longest distance run during the first phase of training was 18 miles, which was performed on two occasions. The second phase of the training program consisted of a taper (reduced training volume) for three weeks. Compared to week 13, the running volume gradually decreased until the total weekly volume for the week before the marathon was decreased by 80%.

During the third week of training, marathon training group participants reported to the Human Performance Laboratory to complete informed consent, medical history questionnaire and the International Physical Activity Questionnaire (IPAQ;

www.ipaq.ki.se). A familiarization trial for vascular ultrasonography was also performed during this visit. Participants reported to the laboratory later in the same week for ultrasonography of the brachial, popliteal and carotid arteries, as well as VO_{2max} and lactate threshold testing. Marathon training participants then returned to the laboratory after the 12^{th} week of training, for IPAQ, VO_{2max}, lactate threshold and ultrasonography measurements.

Control subjects were recruited through a bulk e-mail detailing the research study and the participation requirements. Volunteers for this group reported to the Human Performance Laboratory on three different occasions. During the first visit, they complete the informed consent, medical history questionnaire, IPAQ, and a vascular ultrasonography familiarization trial. Ultrasound measurements of the carotid, brachial and popliteal arteries were performed during the second visit, which was completed by the end of week three of the study. Visit three was completed during weeks 15 and 16 of the study and included follow-up IPAQ and ultrasonography. Participants were also asked to maintain their moderate to vigorous physical activity throughout the duration of the study. Moderate and vigorous activity was defined in accordance with the criteria used on the IPAQ.

Methodology

Ultrasonography and Arterial Dimension Measurements

Participants entered a quiet room with limited lighting and reclined in a supine position for 10 minutes. Following the rest period, right side carotid (1-3cm inferior to the carotid bifurcation), brachial (1-3cm superior to the anticubital fossa), and popliteal

(1-3cm superior to the popliteal fossa) images were obtained with high resolution, B-mode ultrasonography (Mindray, DC-6, 21 Mahwah, NJ) using a probe that was set at 10 MHz.

Analysis of the arterial dimensions was performed with ImageJ software (National Institute of Health; http://rsb.info.nih.gov/ij/). After importing the arterial images into the software, the line tool was used to draw a line parallel to the vessel wall; the line was then rotated 90° and the two ends of the line were adjusted to measure across the tissue areas of interest. All ultrasound measurements preceded exercise testing and were recorded in mm. Intima-media thickness (IMT) was defined as the distance from the edge of the lumen-intima interface to the edge of the media-adventitia interface. Vessel wall thickness (WT) was defined as the distance between the deep lining of the intima (endothelium) and the superficial wall of the adventitia. Lumen diameter (LD) was defined as the distance from one edge of the lumen-intima interface to the edge of the opposing lumen-intima interface. Wall: lumen (W: L) ratio was calculated by dividing the wall thickness measurement by the lumen diameter measurement.

VO_{2max} and Lactate Measurements

A graded exercise test was conducted to determine maximal oxygen uptake (VO_{2max}), submaximal blood lactate concentrations, and lactate threshold. Exercise testing was carried out on a Stairmaster Quinton treadmill (Vancouver, WA). Oxygen consumption (VO_2), respiratory exchange ratio and ventilation were continuously monitored with a (Sensormedics Spectra, Yorba Linda, CA) metabolic cart. Heart rate was also continuously monitored with a portable unit (t1C Suunto, Finland).

The treadmill protocol consisted of two discontinuous phases. Submaximal blood lactate concentrations and lactate threshold were assessed in the first phase. Participants warmed up for five minutes at a speed of 3.5 mph. After the warm-up, the treadmill was set at an individualized velocity that corresponded to the speed that was typically performed during a 60-minute training run. Speed was incrementally increased by 15-20 sec/mile in 3-minute stages. Subjective ratings of exertion (Borg; ACSM Guidelines for Exercise Testing and Prescription 2010) were obtained in the final minute of each stage. At the end of each 3-minute stage, participants straddled the treadmill for a 1-minute rest period. During this time, capillary blood was taken via finger stick and was immediately assessed using an YSI 2300 STAT glucose/lactate analyzer (YSI Incorporated) to determine submaximal blood lactate concentrations and lactate threshold (> 3.5 mmol). Lactate cutoff of 3.5 mmol was used, as opposed to other common lactate threshold indices (i.e., 2.5 mmol and 4.0 mmol), because it was the highest criterion achieved by all participants. Once blood lactate levels exceed lactate threshold the treadmill was stopped and subjects rested for a period of 15 minutes. The speed of running that immediately preceded lactate threshold was compared before and after the marathon training.

Following 15 minutes of passive recovery, VO_{2max} was measured in the second phase of the exercise test. Participants warmed up for three minutes at 3.5 mph. Immediately following the warm up, the treadmill was set at a speed corresponding with the second to last stage of phase one. Stages lasted two minutes and grade was increased by two percent at the beginning of each stage. This protocol was followed until subjects achieved volitional exhaustion.

International Physical Activity Questionnaire

The IPAQ was used to measure time (min per day) spent performing moderate activity and vigorous activity. The IPAQ was used to determine if physical activity was different between the groups and if within group activity was different between the beginning and the end of the study.

Data Analysis

Prior to hypothesis testing, all dependent variables were tested for normality using the Shapiro-Wilkes method. Variables that deviated significantly ($p<.05$) from the normal distribution (e.g., experimental pre-training carotid IMT) and the corresponding variables (e.g., control pre-training carotid IMT as well as control and experimental post-training carotid IMT) were normalized with log transformation. Two by two repeated measures ANOVA with one within subject factor (pre and post measurements) and one between subject factor (training and control groups) was used to identify significant interactions between the groups. When appropriate (time x group interaction; $p<.05$), a paired T-test was used to determine whether or not the dependent arterial dimensions changed over time within either group and an independent T-test was used to assess between group differences. T-test results were adjusted with the Bonferroni correction for multiple comparisons. To assess researcher reliability, test-retest analysis (paired T-test; Pearson product moment correlation and coefficient of variation) was conducted on a separate sample. Two-tailed alpha levels were used for all analyses and all data are presented as mean ± standard deviation.

Chapter III

Manuscript

Abstract

Increased arterial wall thickness has been positively related to cardiovascular disease. Exercise has been found to favorably alter arterial anatomy in the trained limbs, but its effects on untrained limbs and the carotid artery are unclear. Thus, brachial (non-trained limb), popliteal (trained limb) and carotid, wall thickness (WT), wall to lumen diameter ratios (W:L), intima-media thickness (IMT) and lumen diameters (LD) were compared between recreationally active (n=14) and untrained (n=11) subjects before and after, the active subjects participated in a 16 week marathon run training program. Arterial dimensions were measured with B-mode ultrasonography. Pre and post-training VO_{2max} and running speed at 3.5mmol lactate were measured in the marathon training group; VO_{2max} was unaltered, but running speed increased from 7.8 ± 0.7mph to 8.2 ± 0.9 mph ($p=.008$). Time by group interactions were observed for the brachial and popliteal measures ($p<0.05$), but not in the carotid, and no differences were observed in the control group. Prior to the intervention the marathon training group had significantly larger LD in the brachial ($p=.002$) and popliteal arteries ($p=.007$) than the control group; no other pre-training differences were found. Following training, WT was reduced in the brachial (pre = .99 ± .16mm; post = .84 ± .10mm; $p=.007$) and popliteal (pre = .96 ± .09mm; post = .86 ± .11mm; $p=.005$) arteries, and was characterized, in part, by a 0.07mm decrease in brachial IMT ($p=.032$) and a non-significant 0.03mm reduction in popliteal IMT. LD increased in the brachial (pre = 3.38 ± .35mm; post = 3.57 ± .41mm; $p=.015$) and

popliteal (pre = 4.73 ± .48mm; post = 5.11 ± .72mm; p=.002) arteries. These data suggest that arteries in the trained and non-trained limbs are favorably altered by regular exercise and that the alterations may be exercise dose dependent.

Introduction

Exercise training improves vascular function by decreasing overall arterial wall thickness (WT), wall-to-lumen diameter ratios (W:L), intima-media thickness (IMT) and increasing lumen diameter (LD) (O'Leary et al., 1999; Dinenno et al., 2001; Moreau et al., 2006; Thijssen et al., 2007; Thijssen et al., 2011). It is unclear, however, whether these adaptations are localized to the trained limbs or whether they are also manifested in the arteries of non-trained limbs and carotid arteries.

Cross-sectional data indicate that endurance trained runners and triathletes have larger femoral artery LD and smaller femoral IMT and IMT-to-LD ratio when compared to sedentary control subjects (Dinenno et al., 2001). Similarly, Moreau et al. (2006) reported that femoral IMT was lower in endurance trained men and women regardless of age; and, Schmidt-Trucksass et al. (2012) found that femoral LD was larger in runners and cyclist when compared to sedentary controls and subjects with spinal cord injuries. Experimental evidence further suggests that arterial WT (Green et al., 2010; Thijssen et al., 2011), IMT (Dinenno et al., 2001; Spence et al., 2012) and W:L (Green et al., 2010; Thijssen et al., 2011) decrease with training while LD increases (Dinenno et al., 2001; Thijssen et al., 2007; Green et al., 2010, Spence et al., 2012). Together these data demonstrate that conduit arteries of active limbs undergo marked exercise training adaptations.

When compared to trained limbs, regular exercise appears to have a limited effect on the arteries in the non-trained limbs (e.g., arms during cycling or running). In general, the available cross-sectional and longitudinal research suggest that brachial artery WT (Green et al., 2010; Rowley et al., 2011; Rowley et al., 2011) and W:L (Green et al.,

2010; Rowley et al., 2011) are lower among trained subjects, but unlike the adaptations found in the trained limbs, IMT (Thijssen et al., 2007) and LD (Dinenno et al., 2001; Green et al., 2010; Thijssen et al., 2011; Spence et al., 2012) are not different.

The relationship between carotid artery dimensions, especially carotid IMT, and exercise has been studied by numerous researchers, with equivocal results. Cross-sectional studies suggest that carotid IMT is lower in physically active subjects as well as subjects that score higher on fitness tests (e.g., maximal oxygen consumption) (Gando, et al., 2011; Thijssen et al., 2012). Experimental data is less convincing, however, with only limited data showing that carotid IMT is favorably influenced by training (Wildman et al., 2004; Rauramma et al., 2004; Spence et al., 2012). Notably, two multiyear studies (204 and 312 weeks) showed that exercise attenuated an age-related increase carotid IMT (Wildman et al., 2004; Rauramaa et al., 2004) and a third study (Spence et al., 2012) that showed a reduction in carotid IMT included six months of training. These data suggest that alterations in carotid IMT may be related to the duration or volume of training.

A notable weakness within the cited research is a scarcity of data from studies that simultaneously assess arterial WT, W:L, IMT and LD in trained limbs, non-trained limbs and the carotid artery (Spence et al., 2012), thus limiting the ability to determine whether type and volume of exercise causes remodeling in the trained limbs only, or if changes are also manifested in arteries that are peripheral to the trained limbs. Thus, the present study was designed to compare WT, W:L, IMT and LD in trained, non-trained and carotid arteries between recreationally active and untrained subjects and to evaluate whether or not 12 weeks of a progressive marathon training stimulus among the recreationally active subjects leads to arterial remodeling.

It was hypothesized that due to differences in exercise behavior, 1) the training group would have significantly lower WT, W:L and IMT, and greater LD than the control group in the brachial and popliteal arteries, but not the carotid artery prior to 12 weeks of marathon training. It was also hypothesized that 2) there would be a significant group by time interaction characterized by reductions in WT, W:L and IMT, and an increase in LD in all three arteries in the training group with no change in the control group.

Subjects and Methods

Subjects

Fourteen male (n = 5) and female (n = 9) students from a university taught marathon running class and 11 inactive students volunteered to participate in this study. All methods for the study were approved by the James Madison University Institutional Review Board prior to commencement.

Eligibility requirements for the experimental group included the ability to complete a five mile continuous run prior to beginning the training program as well as having not previously run a marathon. After enrolling in the class, participants followed a 16-week marathon-training program. Pre and post-test data were collected before and after a 12-week segment within the training program. The program included four days of training per week and was divided into two phases. The first phase consisted of a 13-week training period that gradually increased the overall training volume by ~140% between week one and week 13. The peak running volume occurred in weeks 12 and 13 with a total of 36 miles run during each of those weeks. The longest distance run during the first phase of training was 18 miles, which was performed on two occasions. The second phase of the training program consisted of a taper (reduced training volume) for three weeks. Compared to week 13, the running volume gradually decreased until the total weekly volume for the week before the marathon was decreased by 80%.

During the third week of training, marathon training group participants reported to the Human Performance Laboratory to complete informed consent, medical history questionnaire and the International Physical Activity Questionnaire (IPAQ; www.ipaq.ki.se). A familiarization trial for vascular ultrasonography was also performed

during this visit. Participants reported to the laboratory later in the same week for ultrasonography of the brachial, popliteal and carotid arteries, as well as VO_{2max} and lactate threshold testing. Marathon training participants then returned to the laboratory after the 12^{th} week of the training intervention, for IPAQ, VO_{2max}, lactate threshold and ultrasonography measurements.

Control subjects were recruited through a bulk e-mail detailing the research study and the participation requirements. During an initial visit to the Human Performance Laboratory, subjects complete the informed consent, medical history questionnaire, and IPAQ. Ultrasound measurements of the carotid, brachial and popliteal arteries were performed during a second visit, which was completed by the end of week three of the study. A third visit was completed during weeks 15 and 16 of the study and included follow-up IPAQ and ultrasonography. Participants were also asked to maintain their moderate to vigorous physical activity throughout the duration of the study. Moderate and vigorous activity was defined in accordance with the criteria used on the IPAQ.

Methodology

Ultrasonography and Arterial Dimension Measurements

Participants entered a quiet room with limited lighting and reclined in a supine position for 10 minutes. Following the rest period, right side carotid (1-3cm inferior to the carotid bifurcation), brachial (1-3cm superior to the anticubital fossa), and popliteal (1-3cm superior to the popliteal fossa) images were obtained with high resolution, B-mode ultrasonography (Mindray, DC-6, 21 Mahwah, NJ) using a probe that was set at 10 MHz.

Analysis of the arterial dimensions was performed with ImageJ software (National Institute of Health; http://rsb.info.nih.gov/ij/). After importing the arterial images into the ImageJ software, the line tool was used to draw a line parallel to the vessel wall; the line was then rotated 90° and the two ends of the line were adjusted to measure across the tissue areas of interest. All ultrasound measurements preceded exercise testing and were recorded in mm. Arterial IMT was defined as the distance from the edge of the lumen-intima interface to the edge of the media-adventitia interface; and, WT was defined as the distance between the deep lining of the intima (endothelium) and the superficial wall of the adventitia. Vessel LD was defined as the distance from one edge of the lumen-intima interface to the edge of the opposing lumen-intima interface; and, W: L was calculated by dividing WT by LD.

VO_{2max} and Lactate Measurements

A graded exercise test was conducted to determine maximal oxygen uptake (VO_{2max}), submaximal blood lactate concentrations, and lactate threshold. Exercise testing was carried out on a Stairmaster Quinton treadmill (Vancouver, WA). Oxygen consumption (VO_2), respiratory exchange ratio and ventilation were continuously monitored with a (Sensormedics Spectra, Yorba Linda, CA) metabolic cart. Heart rate was also continuously monitored with a portable unit (t1C Suunto, Finland).

The treadmill protocol consisted of two discontinuous phases. Submaximal blood lactate concentrations and lactate threshold were assessed in the first phase. Participants warmed up for five minutes at a speed of 3.5 mph. After the warm-up, the treadmill was set at self-selected speed that corresponded to the speed that was typically performed

during a 60-minute training run. Speed was incrementally increased by 15-20 sec/mile in 3-minute stages. Subjective ratings of exertion (Borg; ACSM Guidelines for Exercise Testing and Prescription 2010) were obtained in the final minute of each stage. At the end of each 3-minute stage, participants straddled the treadmill for a 1-minute rest period. During this time, capillary blood was taken via finger stick and was immediately assessed using an YSI 2300 STAT glucose/lactate analyzer (YSI Incorporated, Yellow Springs, OH) to determine submaximal blood lactate concentrations and lactate threshold (> 3.5 mmol). Lactate cutoff of 3.5 mmol was used, as opposed to other common lactate threshold indices (i.e., 2.5 mmol and 4.0 mmol), because it was the highest criterion achieved by all participants. Once blood lactate levels exceed lactate threshold the treadmill was stopped and subjects rested for a period of 15 minutes. The speed of running that immediately preceded lactate threshold was compared before and after the marathon training.

Following 15 minutes of passive recovery, VO_{2max} was measured in the second phase of the exercise test. Participants warmed up for three minutes at 3.5 mph. Immediately following the warm up, the treadmill was set at a speed corresponding with the second to last stage of phase one. Stages lasted two minutes and grade was increased by two percent at the beginning of each stage. This protocol was followed until subjects achieved volitional exhaustion.

International Physical Activity Questionnaire

The IPAQ was used to measure time (min per day) spent performing moderate activity and vigorous activity. The IPAQ was used to determine if physical activity was different

between the groups and if within group activity different between the beginning and the end of the study.

Data Analysis

Prior to hypothesis testing, all dependent variables were tested for normality using the Shapiro-Wilkes method. Variables that deviated significantly ($p < 0.05$) from the normal distribution (e.g., experimental pre-training carotid IMT) and the corresponding variables (e.g., control pre-training carotid IMT as well as control and experimental post-training carotid IMT) were normalized with log transformation. Two by two repeated measures ANOVA with one within subject factor (pre and post measurements) and one between subject factor (training and control groups) was used to identify significant interactions between the groups. When appropriate (time x group interaction; $p<.05$), a paired T-test was used to determine whether or not the dependent arterial dimensions changed over time within either group and an independent T-test was used to assess between group differences. T-test results were adjusted with the Bonferroni correction for multiple comparisons. To assess researcher reliability, test-retest analysis (paired T-test; Pearson product moment correlation and coefficient of variation) was conducted on a separate sample. Two-tailed alpha levels were used for all analyses and all data are presented as mean ± standard deviation.

Results

Evaluation of the test-retest data collected from a separate group of subjects indicated that, pre and post carotid measurements were significantly correlated ($r = 0.981$ to 1.00; $p<0.001$), not significantly different ($t = -0.919$ to 2.049, $p>0.05$) and the coefficient of variation ranged from 0.20 to 2.26%. Pre and post brachial measurements were significantly correlated ($r = 0.947$ to 0.995; $p<0.001$) not significantly different ($t = -2.311$ to -0.396, $p>0.05$) and the coefficient of variation ranged from 0.45 to 2.71%. Pre and post popliteal measurements were significantly correlated ($r = 0.948$ to 0.999; $p<0.001$) not significantly different ($t = -0.016$ to 0.475, $p>0.05$) and the coefficient of variation ranged from 0.32 to1.89%.

There were no significant changes from pre to post measurements in either the marathon training group or the control group in height or weight (Table 3).

Physical Activity and Training Data

Pre training combined moderate and vigorous activity values for the marathon and control groups were 361±210 min/week and 31±56 min/week (between group p=.000), respectively, and post training values were 393±136 min/week and 35±69 min/week (between group p=.000). When the moderate and vigorous activity components were evaluated separately, the marathon group transitioned to spending significantly more time in vigorous exercise than moderate activity by the end of the study (pre moderate: 114±123 vs. post moderate: 70±83; pre vigorous: 247±102 vs. post vigorous: 323±113; p=.038).

In the marathon training group, VO_{2max} (ml/kg/min; L/min) did not differ between pre and post measurements. Running speed at lactate threshold increase significantly (p=.008) and lactate concentrations at a running speed that equated to average speed during a 60 min training run decreased significantly (p=.001) (Table 4).

Arterial Thicknesses, Diameters and Ratios

Carotid Artery

Pre-training carotid arterial measurements were not different between the groups. Likewise, pre- and post-carotid artery WT, IMT, LD, and W:L were not different in either group (Table 5).

Brachial Artery

Prior to training, the marathon training group had significantly larger brachial LD (p=.002) than the control group: no other pre-training between group differences were found. There were no significant changes in the brachial artery measurements within the control group. Marathon training decreased brachial artery WT (p=.007) and increased LD (p=.015). These combined changes were reflected by a significant reduction in W:L (p=.000). IMT of the brachial artery tended to decreased in the training group (p=.032) (Table 5).

Popliteal Artery

Prior to training, the marathon training group had significantly larger LD (p=.007) than the control group; no other pre-training between group differences were found.

There were no significant changes in the popliteal artery measurements within the control group. Marathon training decreased popliteal WT (p=.005) and increased LD (p=.007). There was also a significant reduction in W:L (p=.000). IMT of the popliteal artery did not change in the training group (p=.260) (Table 5).

Discussion

Physical activity and training

Arterial dimensions were compared between recreationally active college students who participated in a marathon run training program and sedentary control subjects. The IPAQ data showed that the marathon training group was involved in significantly more moderate and vigorous activity than the control group before (p=.000) and after the training period (p=.000). The IPAQ data also showed that the amount of vigorous activity increased significantly within the marathon training group by the end of the training program (p=.038). Although VO_{2max} did not change in the training group, a training adaptation was evident based on increased running speed at lactate threshold (p=.008) and decreased lactate concentrations during a constant running speed (p=.001) (Table 4). These data establish that the activity levels between the two groups were significantly different before and after the training intervention and that the marathon training group's treadmill running performance improved as a result of the training program.

Pre-training between group comparisons

The marathon training group had significantly larger LD in the brachial (p=.002) and popliteal (p=.007) arteries than the control group prior to training, whereas no differences were observed in the carotid LD. Pre-training WT, W:L and IMT measurements were not different between the groups in any of the arteries. The LD data is consistent with cross-sectional data presented by Rowley et al. (2011) who found that LD in the brachial and femoral arteries of athletes was greater than inactive age-matched controls, but that carotid LD was not different. In contrast to the present study, Rowley et

al. (2011) also reported that arterial WT was lower in athletes when compared to controls. There are several possible explanations for this discrepancy (Table 5).

Based on their research, Rowley et al. (2011) proposed that exercise induced increases in LD are caused by local mechanisms (i.e. shear induced NO production) and that reductions in WT are mediated by systemic mechanisms (e.g., arterial pressure). Tinken et al. (2008) also demonstrated that the time courses of functional and anatomical arterial adaptations to exercise training are different, even with identical training. Given the comparatively unsupervised nature of the exercise within the training group prior to initiating the marathon training program as well as the data suggesting that arterial adaptations may be dependent on the type and time course of the stimulation, it is not surprising that LD was found to be different between the two groups while WT was not (Table 5). Other evidence shows that WT increases with age (Moreau, et al. 2006; van den Munckhof, et al. 2012) and that the increases in WT are proportionally greater than age-related changes in LD (van den Munckhof, et al. 2012). These data further suggest that younger individuals with thinner vessel walls may experience a proportionally greater increase in LD compared to WT with training.

The between group differences found in the IPAQ and arterial dimension data provides evidence of a mild effect of physical activity that existed prior to beginning the marathon training program. In combination with the training effect seen the marathon training group (discussed below), the initial between group differences suggest that arterial adaptations are dose-dependent.

Adaptations to training

Consistent with previous research we observed no training adaptations in the carotid artery (Schmidt-Trucksass et al., 2000; Rowley et al., 2011; Rowley et al., 2011; Spence et al., 2012) but found significant remodeling in the popliteal artery, or trained limb, of the marathon training group (Schmidt-Trucksass et al., 2000; Rowley et al., 2011; Green et al., 2010; Thijssen et al., 2011; Spence et al., 2012; Dinenno et al., 2001) (Table 5). Popliteal adaptations included reductions in WT (-9.98%) and W:L (-16.5%) as well as an 8.2% increase in LD. No change was found in the popliteal IMT in the marathon training group.

Similar to Green et al. (2010), but in contrast to others (Thijssen et al., 2007; Dinneno et al., 2001; Green et al., 2010; Thijssen et al., 2011; Spence et al., 2012), we found that brachial artery, or non-trained limb, dimensions adapted to the 12 weeks of training (Table 5). Significant reductions were seen in WT (-15.3%) and W:L (-19.4%) , while LD increased 5.7% and IMT trended toward a significant reduction (-14.4%; $p = .032$). These results indicate that arterial adaptations to exercise training can be manifested in the non-trained limb as well as the trained limb. The data further suggest that adaptations may be dependent on training volume. This interpretation is supported by the evidence that the marathon training group had greater LD in the brachial and popliteal arteries than the sedentary control group at the beginning of the study and the fact that LD further increased when training volume was increased. Our observations of increased LD in the brachial and popliteal arteries of the training group are consistent with research done by Green et al. (2010) and Thijssen et al. (2007). Green et al. (2010) found brachial LD to increase by approximately 4% and popliteal LD to increase by about 12%.

Thijssen et al., (2007) found femoral LD to increase by about 7% with training. The reduction in WT and W:L in the brachial and popliteal arteries observed in this study are also consistent with the work done by Green et al. (2010) who reported reductions in brachial WT (14%) and W:L (17%) as well as reductions in popliteal WT (10%) and W:L (14%).

Several mechanisms appear to contribute to arterial changes (Thijssen, et al., 2011). Included among these are the endothelial derived NO-dilator system, exercise induced variations in systemic blood pressure as well as adaptations to oxidative stress and localized inflammation.

In the context of the present study, the role of NO is of particular interest given its responsiveness to variations in blood flow. Specifically, as blood flow increases, there is an increase in the shear stress acting on the vessel. Shear stress is a tractive force exerted parallel to the intimal wall and is characterized by an increase in blood flow that is proportionally greater than changes in vessel diameter (Niebauer and Cooke, 1996). With the increases in arterial blood flow that accompanies exercise, shear stress stimulates activity of the NO-dilator system, including the up-regulation of endothelial-derived NO synthase, to buffer the increased shear (Maiorana et al., 2003). Although the precise mechanisms have yet to be elucidated, the increased activity of the NO-dilator system is associated with the modulation of platelet, macrophage and endothelial derived growth factors that favors increased LD and reductions in arterial WT. This arterial remodeling continues until the shear stress is normalized and the NO mechanism can return back to its baseline activity (Maiorana et al., 2003).

The simultaneous assessment of the carotid, brachial and popliteal arteries as performed in this study combined with blood flow and endothelial function data from other research provides a basis for explaining the potential role of shear stress and the NO-dilator system in arterial remodeling. There is clear evidence that blood flow to different regions of the body is varied during exercise (Ahlborg et al., 1975; Bangsbo et al., 1995) and that it is further influenced by exercise mode (Calbet et al., 2004) and intensity (Goto et al., 2003; Tanaka, et al., 2006). While no data was found regarding blood flow to the legs and arms during running, Calbet et al. (2004) showed that leg-only cross-country skiing, lead to a seven to eight-fold increase in femoral blood flow and a two to three-fold increase in subclavian blood flow. Similar to running, leg-only cross-country skiing requires that the legs support the body weight in the skiing motion, as the arms actively counterbalance the motion of the legs. Other studies show that when the arms are immobilized during leg exercise, blood flow in the brachial artery increases as much as four-fold (Green et al., 2002; Green et al., 2005; Tanaka et al., 2006) and that the higher flow is adequate to stimulate the NO-dilator system (Miyachi et al. 2001; Green et al., 2002). In contrast, blood flow to the carotid artery during exercise increases to a lesser degree. Hellstrom et al. (1996), for example, found that the maximum change in blood flow to the head during exercise is 30-40% higher than resting values.

Additionally, Tanaka et al. (2006) found that blood flow to the active limbs increases in proportion to exercise intensity, while Goto et al. (2003) showed that blood flow within a range of intensities is needed to stimulate the NO-dilator system and potentially alter arterial dimensions. Goto et al. (2003) reported that moderate intensity exercise (50% Vo_{2max}) is within the range required to enhance endothelial function in the

non-training limb. On the other hand, these researchers (Goto et al., 2003) reported that low intensity exercise (25% Vo_{2max}) failed to stimulate the NO-dilator system, and any stimulatory effect associated with exercise at higher intensities (75+% Vo_{2max}), may be countered by an inflammatory response.

Given that leg exercise substantially increases blood flow in the legs and arms, whether the arms are immobilized (Green et al. 2002) or not (Calbet et al. 2004), and that flow to the arms is adequate to invoke endothelial-derived dilation (Green et al. 2002), it is plausible that the brachial and popliteal adaptations in LD, WT, W:L found in the marathon training group were mediated by the NO-dilator system. Although some researchers (Dinenno et al., 2001; Thijssen et al., 2007) report that leg exercise is not associated with alterations in brachial artery dimensions, we propose that the adaptations found in the present study were a function of repeated bouts of moderate to high intensity exercise that were performed during 12 weeks of training. This exercise volume may have exceeded that used in studies that failed to show brachial artery adaptations to lower limb exercise training (Dinenno et al., 2001; Thijssen et al., 2007). Moreover, the fact that LD, but not WT, was different between the two groups prior to training and that LD, WT and W:L changed in the training group supports this position.

Despite the significant body of evidence indicating the NO-dilator system's role in regulating arterial anatomy, the cyclical change in blood pressure that accompanies exercise training has not been ruled out as a mechanism responsible for these changes. Chronic elevations in transluminal pressure against the arterial wall, as seen in hypertension, activates pro-atherogenic endothelial cell phenotypes known to contribute to arterial wall thickening (Gomez-Cabrera et al., 2008). Exercise training, however, is

associated with transient and cyclical increases in pressure; and, it has been proposed that the regulation of pro-atherogenic and anti-atherogenic genes is favorably altered by these cyclical variations in pressure (Newcomer et al., 2011). Testing this theory is confounded by the fact that the exercise stimulus leading to increases in pressure are also associated with increases in blood flow and shear stress (Newcomer et al., 2011). In one recent study, however, Rowley et al. (2011) suggested that differences in brachial and femoral WT between elite squash players and sedentary subjects combined with a lack of any difference between arterial WT in the dominant and non-dominant arms of squash players supports the systemic mechanism theory. The data from Rowley et al. (2011) supports the need for more research designed to better explain the role that blood pressure plays in arterial remodeling.

A particularly intriguing finding in this study was that following the marathon training brachial IMT in the experimental group trended toward significance ($p=.032$) while popliteal IMT remained unchanged ($p=.260$). This result is in contrast with data presented by others (Dinenno et al., 2001; Green et al., 2010; Spence et al., 2012) showing that IMT decreased in the trained limb as a result of exercise training. It is possible that the discrepancy is due to localized inflammation in the lower extremities associated with a relatively high volume of training (36 miles, inclusive of an 18 mile run) that occurred during the week prior to data collection. In response to this training load, the subjects may have experienced enough micro-trauma in the legs to stimulate localized inflammation. This explanation is supported by limited data (Nemet et al., 2002; Goto et al., 2003) and constitutes an area of research that has been identified as one in need of further investigation (Thijssen et al., 2012).

An alternate explanation for the absence of change in popliteal IMT may be related to variations in oscillatory pattern of blood flow in limbs during exercise. Thijssen et al. (2009) and Tinken et al. (2009) demonstrated that the NO-dilator activity is directly related to ratio of antegrade to retrograde flow; and vessel occlusion reduces the ratio in favor of retrograde flow and diminished NO-dilator activity. The reduction in NO-dilator activity is proportional to the degree of occlusion, and even a mild occlusion (i.e., cuff pressure at 25 mmHg) is adequate to stimulate a significant change in the ratio and NO-dilator activity. Based on this data, it seems plausible that during the loading phase of the running stride, occlusion of the conduit arteries in the leg would favor a reduction in the antegrade to retrograde flow and perhaps attenuate NO-dilator activity and any related effect on IMT.

Summary

Simultaneous comparison of WT, W:L, IMT and LD in carotid, brachial and popliteal arteries shows that LD is different between young, recreationally active and sedentary subjects and that arterial dimensions in the trained and non-trained limb are further altered with marathon training. The combined evidence suggests that these adaptations are dependent on training volume. These data indicate that further study is needed to explore the interactions between the antegrade to retrograde flow ratio and the NO-dilator system as well as the role that localized inflammation may accompany intense training.

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Table 3. Subject Characteristics

	Trained (n=14)		Control (n=11)	
	Pre	Post	Pre	Post
Age (yrs)	20.4 ±1.6		19.4 ±1.1	
Height (m)	1.72±0.08	1.71±0.08	1.63±0.06	1.63±0.05
Weight (kg)	65.48±8.78	64.07±7.49	64.92±10.87	66.07±10.94

Table 4. Treadmill Speed, Lactate and VO_2 in Trained Subjects

	Pre	Post
Lactate @ mean mph(7.54±0.69)	2.54±.56	1.87±.65*
Treadmill speed (mph) @ 3.5 mmol lactate	7.75±.72	8.15±.86*
VO_{2max} (ml/kg/min)	43.32±5.40	44.11±5.0
VO_{2max} (L/min)	2.80±.56	2.82±.49
p<.01 within group before and after training		

Table 5. Arterial Wall Thickness, Diameter and Ratio

	Trained (n=14)		Control (n=11)	
	Pre	Post	Pre	Post
Carotid				
WT (mm)	1.02±.07	.91±.16	.98±.06	.92±.06
IMT (mm)	.52±.08	.48±.10	.55±.05	.47±.09
LD (mm)	5.85±.86	5.90±.63	5.86±.97	5.74±.88
W:L	.18±.030	.16±.023	.17±.031	.16±.023
Brachial				
WT (mm)	.99±.16**	.84±.10	.92±.14	.92±.008
IMT (mm)	.49±.09	.42±.08	.46±.10	.47±.07
LD (mm)	3.38±.35* **	3.57±.41	2.98±.28	2.88±.29
W:L	.29±.044**	.24±.034	.31±.043	.32±.038
Popliteal				
WT (mm)	.96±.09**	.86±.11	.89±.11	.88±.07
IMT (mm)	.47±.05	.44±.07	.46±.06	.48±.08
LD (mm)	4.73±.48* **	5.11±.72	4.13±.51	4.15±.48
W:L	.20±.027**	.17±.027	.22±.023	.21±.022

WT, wall thickness; IMT, intima-media thickness; LD, lumen diameter; W:L, wall to lumen ratio.
*p<.01 between group before training
**p<.025 within group before and after training

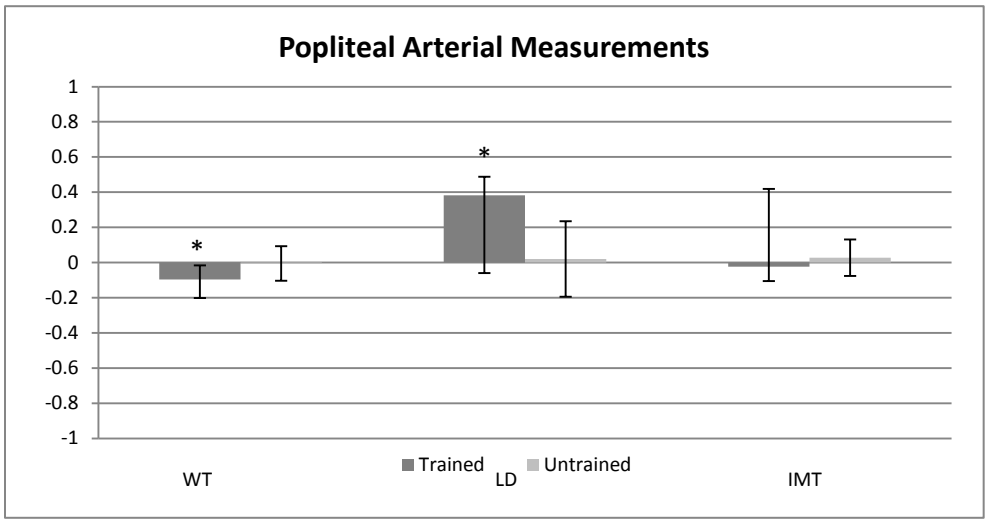


Figure 1. Changes (mm) in popliteal vessel dimensions. Wall thickness (WT), Lumen diameter (LD), Intima-media thickness (IMT). All data represented as mean±.standard deviation. *p<.025

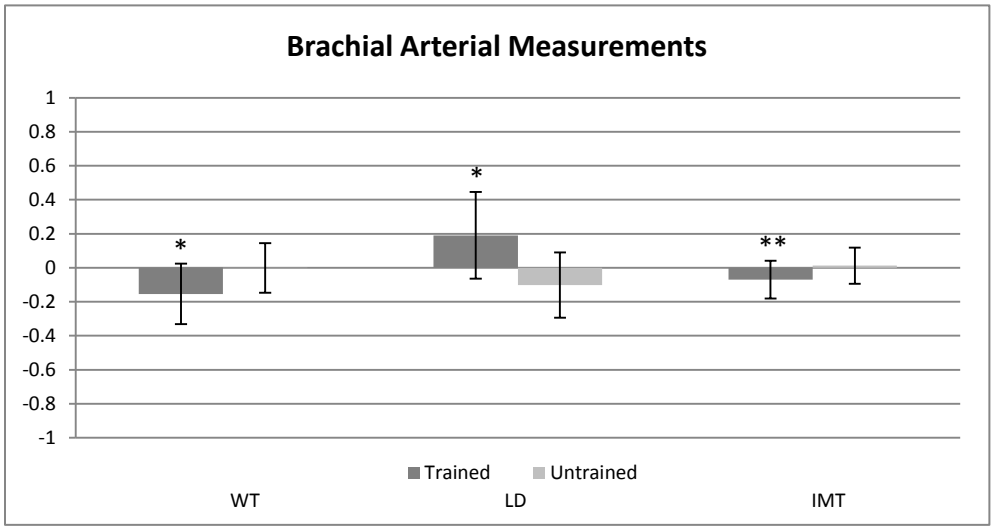


Figure 2. Changes (mm) in brachial vessel dimensions. Wall thickness (WT), Lumen diameter (LD), Intima-media thickness (IMT). All data represented as mean±.standard deviation. *p<.025, **p=.032

Appendix A

James Madison University
Department of Kinesiology
Informed Consent
Marathon Training Subjects

Purpose

You are being asked to volunteer for a study conducted by Dr. Todd, Dr. Luden, Nicole Hafner and Corey Greever titled "Aerobic, skeletal muscle, and vascular adaptations to marathon run training with and without concurrent resistance training". The primary aims of this study are to determine if marathon training alters the diameter and thickness of the vessels in your neck (carotid), arm (brachial) and leg (popliteal), blood flow mechanics in your brachial and popliteal arteries, skeletal muscle architecture (shape and size) of your calf and thigh, and your cardiovascular physiology.

Experimental Procedures

You will be asked to report to James Madison University's Human Performance Laboratory (Godwin 209) on three occasions. Specifically, you will be asked to report to the laboratory twice at the beginning of the marathon-training program, and once more towards the end of the marathon-training program. Visits 1 (1 hr) and 2 (1.5 hrs) will take a combined 2.5 hrs and visit 3 will require 2 hrs, for a total time commitment of approximately 4.5 hrs. Detailed information for each of these trials is provided below:

Visit 1 – Week of September 12th

Prior to any data collection, you will be asked to complete a health history questionnaire to ensure that you meet the study criteria and that you do not have any risk factors that would prevent you from performing heavy exercise, although this is unlikely due to your participation in the GKIN 100-marathon class. In the process of filling out these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and weight and you will be asked to fill out the International Physical Activity Questionnaire (IPAQ). The IPAQ is used to measure time spent sitting, walking, performing moderate activity and vigorous activity. You will also be asked to abide by some guidelines concerning vitamin supplementation, medication use, caffeine use, previous exercise and fasted state so that measurements obtained are the most accurate (see attached form). Lastly, you will be asked to fill out a form ranking how often you eat certain foods. The purpose of this is because some foods eaten often can have affects on the vascular system.

Then, to familiarize you with the vascular assessment procedures, you will be asked to undergo an ultrasound and flow mediation dilation evaluation of your brachial artery. This non-invasive procedure involves lying down and relaxing in a cool dark room while the investigator images the artery using a 5-10 MHz ultrasound scanner (Mindray DC-6). Once the image is saved a flow mediated dilation measurement will be taken. This involves the placement of a blood pressure cuff distal to the artery being imaged and inflated to 250 mmHg for 5 minutes. After this time, the cuff will be deflated and measurements of dilation will be recorded for 2 minutes.

Following the vascular familiarization trial, you will be asked to undergo a DEXA scan for measures of body composition (percent body fat, lean body mass, and bone mineral density). You will be asked to lie on your back completely still, while breathing normally and closing your eyes while the scan is in progress. The entire scan lasts approximately 6 minutes.

Finally, you will be asked to perform a muscle function test. Following a 5-minute treadmill warm-up at a self-selected walking speed, you will be positioned in a custom-built leg extension machine equipped with a force transducer. When prompted, you will perform a maximal leg extension against the padded stationary leg extension bar. The force produced by you will be processed by the transducer, recorded, and stored in a computer for analysis.

Visit 2 – Week of September 12th

At least 24 hrs following visit 1, you will be asked to report to the laboratory for visit 2, in which you will be asked to perform a treadmill test and measures of vascular physiology and skeletal muscle architecture. Upon reporting to the lab, you will be asked to lie down and relax in a cool dark room while the investigator images your arteries (neck, leg, and arm) using the ultrasound scanner. Once the image is saved, a flow mediated dilation measurement will be taken. This involves the placement of a blood pressure cuff distal to the artery being imaged and inflated to 250 mmHg for 5 minutes. After this time, the cuff will be deflated and measurements of dilation will be recorded for 2 minutes. Immediately following the vascular assessment, ultrasound measurements of your vastus lateralis (outside quadriceps muscle) and lateral gastrocnemius (outside calf muscle) will be obtained. This will require you to stand upright with muscles relaxed while the investigator indentifies and scans the two muscles using a 5-10 MHz ultrasound scanner (Mindray DC-6). Once the ultrasound is complete, upon your permission, investigators will mark the ultrasound sites with a medical grade pen. This marking is important because it will identify the exact sites to be used for the post-measurement. There will not be any negative consequences if you prefer not to have the marks on your legs.

Immediately following the ultrasound measurements, you will be asked to perform a treadmill running test. The test is designed to assess your cardiovascular fitness. To do this, the initial treadmill speed will be subjectively determined during a self-selected 5-minute warm-up. You will be instructed to select a speed that you could maintain during a prolonged run of "easy to moderate" intensity. Following the warm-up you will run at this pace for 3-minutes. You will then dismount the treadmill and a drop of blood will be obtained through a finger lancet and analyzed for blood lactate during a 1-minute rest period. These three-minute stages will continue (estimate approximately 6-8 samples), increasing .4 mph in speed, until you have exceeded your lactate threshold (moderate to vigorous intensity). The treadmill speed that elicits your lactate threshold will then stay constant and the treadmill grade will increase 2 percent every 2 minutes until you request to stop or are unable to continue running. The test is no more vigorous than what you will perform during their marathon training intervention

Metabolic measurements such as oxygen uptake and ventilation will be measured during the treadmill test using a metabolic cart. To do this, you will be asked to breathe through a mouthpiece/breathing apparatus that collects your expired breath during the entire duration of the test. You will also be asked to provide subjective ratings of your exertion level at various time points throughout the exercise protocol. You will do this by pointing

to your corresponding level of exertion (rated numerically from 6-20) on a Borg RPE scale. Your heart rate will also be measured using a Polar heart rate monitor that will be worn around your chest during each exercise session.

Visit 3 – Week of November 28th

You will be asked to return to the laboratory to complete post-measures of IPAQ, DEXA, food intake form, FMD checklist, ultrasonography (vascular physiology including flow mediated dilation and skeletal muscle architecture), muscle strength test, and treadmill testing.

Risks

Ultrasonography: Ultrasonography is a non-invasive and risk-free procedure. There are no known adverse effects.

Treadmill Testing: According to the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, the risk associated with maximal testing for individuals categorized as "low risk" is very minimal, and physician supervision and approval is not necessary. The conditions that the exercise sessions are to take place are likely safer than your typical exercise environment. If you do not meet the ACSM criteria for "low risk", you will not be permitted to participate in the study. A physician will be available by pager if the need for medical attention arises throughout the study period. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, at least one of the listed investigators will be present during all exercise sessions, and all are CPR certified. The exercise protocol may result in minor-moderate levels of muscle soreness and fatigue for 1-2 days following each exercise session. Since running is a largely eccentric exercise it is possible that you will experience soreness for up to 48 hours post exercise. It should be mentioned though that the test is no more rigorous than what you will be performing during the marathon training intervention and the risk for soreness is minimal.

Finger Stick Blood Sampling: The risks associated with obtaining small samples of blood via finger-sticks are minimal but include bruising and discomfort for 24 to 48 hours and infection. The risk for infection is small and will be minimized by the use of sterile methods, including the use of sterile alcohol pads, sterile gauze, and band-aids.

Muscle Strength Testing: The risks of muscle strength testing include soreness from exertion 24-72 hours post and potential lightheadedness or loss of consciousness if correct breathing technique is not utilized. These risks will be minimized by instructing and emphasizing proper breathing technique.

Flow Mediated Dilation: The risks of flow mediated dilation measurements include discomfort often described as your arm or leg is "falling asleep"; there is a temporary reduction or loss of feeling because the vessel is occluded for 5 minutes.

DEXA: The risk of DEXA is exposure to low dose radiation associated with the x-ray scan. According to the manufacturer's specifications, whole body DEXA analysis exposes participants to 1.5 mrem of radiation. The exposure to radiation during a single chest x-ray is more than 3 times greater than radiation from DEXA. Also, background radiation from DEXA is about equal to the amount of radiation one experiences during a flight from New York to London. If you are pregnant or think you may be pregnant, you should **not** participate in the DEXA scan. Further, the effects of radiation are

accumulative. Thus, if you are concerned about your previous levels of radiation exposure, please communicate these concerns will the investigative team.

Benefits

You will receive a free VO2max assessment and body composition assessment (DEXA), which includes measures of percent body fat, lean mass and bone mineral density. In addition, you will gain valuable information about your movement efficiency, muscle physiology, and vascular health. This knowledge may aid your training and performance. Participation in this novel research project will also contribute to our understanding of physiological adaptation to marathon training with and without concurrent RE.

Inquiries

If you have any questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact Nicole Hafner at hafnernm@dukes.jmu.edu or Cory Greever at greev2cj@dukes.jmu.edu. In the case of any immediate concerns or adverse reactions during the study, call Dr. Luden at (540) 568-4069 or Dr. Todd at (540) 209-2001.

Confidentiality

The results of this research project will be presented at regional and national conferences and in peer-reviewed exercise science journals. All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All de-identified data will be kept secured in a locked cabinet and will remain there indefinitely. Final aggregate results will be made available to participants upon request.

Freedom of Consent

Your participation is entirely voluntary. Your decision to participate or not will not have any influence on your GKIN 100 grade or alter your standing in the class. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____	_____
Name of Subject (Printed)	Name of Researcher (Printed)
_____	_____
Name of Subject (Signed)	Name of Researcher (Signed)
_____	_____
Date	Date

Appendix B

James Madison University
Department of Kinesiology
Informed Consent

Control Subjects

Purpose
You are being asked to volunteer for a study conducted by Dr. Todd, Dr. Luden, Nicole Hafner and Corey Greever titled "Aerobic, skeletal muscle, and vascular adaptations to marathon run training with and without concurrent resistance training". The primary aims of this study are to determine if marathon training alters the diameter and thickness of the vessels the neck (carotid), arm (brachial) and leg (popliteal), blood flow mechanics in the brachial and popliteal arteries, or skeletal muscle architecture (shape and size) of the calf and thigh. To verify that our data are accurate and not the result of artifact in our measurements, it is critical that we include control subjects that should not experience any physiological changes resulting from alterations in physical activity habits over the course of our study.

Participants will be asked to report to the lab on three different occasions, the wk of 9.19.11 (visits 1 and 2) and the wk of 12.5.11 (visit 3). For visit 1 (50 minutes), subjects will report to the laboratory to complete the informed consent, medical history questionnaire, IPAQ, vascular ultrasonography of the brachial artery, and DEXA. Visit 2 will include both vascular and skeletal muscle ultrasonography (40 minutes). Visit 3 will include IPAQ, vascular and skeletal muscle ultrasonography, and a DEXA (50 minutes).

Experimental Procedures
You will be asked to report to James Madison University's Human Performance Laboratory (Godwin 209) on three occasions. Visits 1 (50 min) and 2 (40 min) will take a combined 1.5 hrs and visit 3 will require another 50 min, for a total time commitment of approximately 2.5 hrs. Detailed information for each of these trials is provided below:

Visit 1 – Week of September 19th

Prior to any data collection, you will be asked to complete a health history questionnaire to ensure that you meet the study criteria. In the process of filling out these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and weight and you will be asked to you are approved to participate in the study, after completing the health history questionnaire, you will be asked to fill out the International Physical Activity Questionnaire (IPAQ). The IPAQ is used to measure time spent sitting, walking, performing moderate activity and vigorous activity. You will also be asked to abide by some guidelines concerning vitamin supplementation, medication use, caffeine use, previous exercise and fasted state so that measurements obtained are the most accurate (see attached form). Lastly, you will be asked to fill out a form ranking how often

you eat certain foods. The purpose of this is because some foods eaten often can have affects on the vascular system.

Then, to familiarize you with the vascular assessment procedures, you will be asked to undergo an ultrasound and flow mediation dilation evaluation of your brachial artery. This non-invasive procedure involves lying down and relaxing in a cool dark room while the investigator images the artery using a 5-10 MHz ultrasound scanner (Mindray DC-6). Once the image is saved a flow mediated dilation measurement will be taken. This involves the placement of a blood pressure cuff distal to the artery being imaged and inflated to 250 mmHg for 5 minutes. After this time, the cuff will be deflated and measurements of dilation will be recorded for 2 minutes.

Following the vascular familiarization trial, you will be asked to undergo a DEXA scan for measures of body composition (percent body fat, lean body mass, and bone mineral density). You will be asked to lie on your back completely still, while breathing normally and closing your eyes while the scan is in progress. The entire scan lasts approximately 6 minutes.

Finally, you will be asked to perform a muscle function test. Following a 5-minute treadmill warm-up at a self-selected walking speed, you will be positioned in a custom-built leg extension machine equipped with a force transducer. When prompted, you will perform a maximal leg extension against the padded stationary leg extension bar. The force produced by you will be processed by the transducer, recorded, and stored in a computer for analysis.

Visit 2 – Week of September 19th

At least 24 hrs following visit 1, you will be asked to report to the laboratory for visit 2, in which you will be asked to undergo measures of vascular physiology and skeletal muscle architecture. Upon reporting to the lab, you will be asked to lie down and relax in a cool dark room while the investigator images your arteries (neck, leg, and arm) using the ultrasound scanner. Once the image is saved, a flow mediated dilation measurement will be taken. This involves the placement of a blood pressure cuff distal to the artery being imaged and inflated to 250 mmHg for 5 minutes. After this time, the cuff will be deflated and measurements of dilation will be recorded for 2 minutes. Immediately following the vascular assessment, ultrasound measurements of your vastus lateralis (outside quadriceps muscle) and lateral gastrocnemius (outside calf muscle) will be obtained. This will require you to stand upright with muscles relaxed while the investigator identifies and scans the two muscles using a 5-10 MHz ultrasound scanner (Mindray DC-6). Once the ultrasound is complete, upon your permission, investigators will mark the ultrasound sites with a medical grade pen. This marking is important because it will identify the exact sites to be used for the post-measurement. There will not be any negative consequences if you prefer not to have the marks on your legs.

Visit 3 – Week of November 28th

You will be asked to return to the laboratory to complete post-measures of IPAQ, DEXA, food intake form, FMD checklist, ultrasonography (vascular physiology including flow mediated dilation and skeletal muscle architecture), muscle strength test, and treadmill testing.

Risks

Ultrasonography: Ultrasonography is a non-invasive and risk-free procedure. There are no known adverse effects.

Muscle Strength Testing: The risks of muscle strength testing include soreness from exertion 24-72 hours post and potential lightheadedness or loss of consciousness if correct breathing technique is not utilized. These risks will be minimized by instructing and emphasizing proper breathing technique.

Flow Mediated Dilation: The risks of FMD measurements include discomfort often described as your arm or leg is "falling asleep"; there is a temporary reduction or loss of feeling because the vessel is occluded for 5 minutes.

DEXA: The risk of DEXA is exposure to low dose radiation associated with the x-ray scan. According to the manufacturer's specifications, whole body DEXA analysis exposes participants to 1.5 mrem of radiation. The exposure to radiation during a single chest x-ray is more than 3 times greater than radiation from DEXA. Also, background radiation from DEXA is about equal to the amount of radiation one experiences during a flight from New York to London. If you are pregnant or think you may be pregnant, you should **not** participate in the DEXA scan. Further, the effects of radiation are accumulative. Thus, if you are concerned about your previous levels of radiation exposure, please communicate these concerns will the investigative team.

Benefits

You will receive a free body composition assessment (DEXA), which includes measures of percent body fat, lean mass and bone mineral density. In addition, you will gain valuable information about your muscle physiology and vascular health. Participation in this novel research project will also contribute to our understanding of physiological adaptation to marathon training with and without concurrent RE.

Inquiries

If you have any questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact Nicole Hafner at hafnernm@dukes.jmu.edu or Cory Greever at greev2cj@dukes.jmu.edu. In the case of any immediate concerns or adverse reactions during the study, call Dr. Luden at (540) 568-4069 or Dr. Todd at (540) 209-2001.

Confidentiality

The results of this research project will be presented at regional and national conferences and in peer-reviewed exercise science journals. All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All de-identified data will be kept secured in a locked cabinet and will remain there indefinitely. Final aggregate results will be made available to participants upon request.

Freedom of Consent

Your participation is entirely voluntary. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____	_____
Name of Subject (Printed)	Name of Researcher (Printed)
_____	_____
Name of Subject (Signed)	Name of Researcher (Signed)
_____	_____
Date	Date

Appendix C

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all *true* statements.

History

You have had:

___ A heart attack
___ Heart surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Heart failure
___ Heart transplantation
___ Congenital heart disease

Symptoms

___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness.
___ You experience dizziness, fainting, blackouts.
___ You take heart medications.

*If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.*

Other health issues

___ You have diabetes
___ You have or asthma other lung disease.
___ You have burning or cramping in your lower legs when walking short distances.
___ You have musculoskeletal problems that limit your physical activity.
___ You have concerns about the safety of exercise.
___ You take prescription medication(s).
___ You are pregnant.

Cardiovascular risk factors

___ You are a man older than 45 years.
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal.
___ You smoke, or quite within the previous 6 mo.
___ Your BP is greater than 140/90.
___ You don't know your BP.
___ You take BP medication.
___ Your blood cholesterol level is >200 mg/dL.
___ You don't know your cholesterol level.
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week).
___ You are more than 20 pounds overweight.

*If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a **professionally qualified exercise staff** to guide your exercise program.*

___ None of the above is true.

You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.

Balady et al. (1998). AHA/ACSM Joint Statement: Recommendations for Cardiovascular Screening, Staffing, and Emergency Policies at Health/Fitness Facilities. *Medicine & Science in Sports & Exercise*, 30(6). (Also in: *ACSM's Guidelines for Exercise Testing and Prescription*, 7th Edition, 2005. Lippincott Williams and Wilkins http://www.lww.com)

www.acsm-msse.org/pt/pt-core/template-journal/msse/media/0698c.htm

Appendix D

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

Long Form: Last 7 Days, Self-Administered Format

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ ***Yes***

☐ **No** → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do

moderate physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. *How much time did you usually spend on one of those days* ***walking*** *from place to place?*

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating

Appendix E

Please Complete the Following:
Sex: Male Female (circle one)
Age (yrs):
Height (inches):
Weight (lbs):
Average Exercise Habits over the Past 3 Months:

Avg. # days of exercise per week:

Avg. # of days of aerobic exercise per week:

Do you have a muscle or joint injury that precludes the completion of the exercise protocol?

Appendix F

FMD Checklist/Form

1. **Are you currently taking any daily vitamins? If so, please list what type and when you take them.**

Vitamin	**Time of Day Taken**

2. **Please list any medications you are currently taking and what time of day you take them.**

Medication	**Time of Day Taken**

It is imperative that the following guidelines be followed in order for the most accurate data to be collected:

1. **Take vitamins at the same time every day that you take them.**

2. **Do NOT take any aspirin/ibuprofen within 3 days to data collection.**

3. **12 hours or more before data collection refrain from:**

    - **Smoking or using any tobacco products or being exposed to cigarette smoke**
    - **Do NOT drink or eat anything with caffeine**
    - **Do NOT consume any alcohol**
    - **Do NOT engage in any exercise**
    - **Do NOT eat or drink anything before data collection**

Appendix G

FMD Checklist

Please mark the following with either a Y (yes) or N (no):

- **Taken all of your vitamins at the same time of day each day _____**
- **Refrained from using aspirin/ibuprofen for last 3 days _____**
- **Within the last 12 hours have you:**

    1) **Used any tobacco products _____**
    2) **Eaten/drank anything w/ caffeine _____**
    3) **Consumed any alcohol _____**
    4) **Engaged in any exercise _____**
    5) **Eaten/drank anything _____**

Appendix H

L-Arginine Rich Food Intake Questionnaire

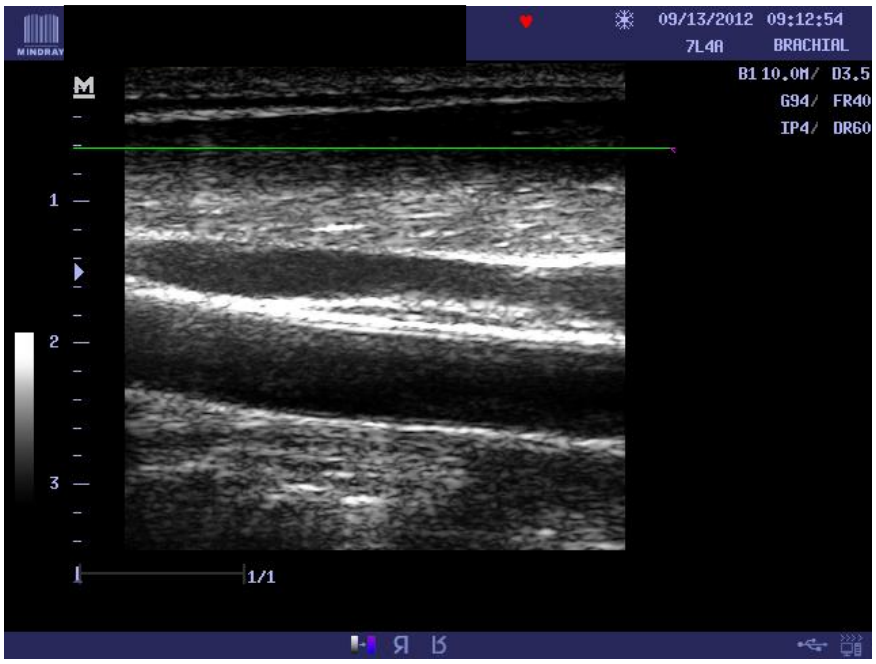
For each of the following items, circle the number that is appropriate:

1 = less than I typically eat in a month;
2 = about the same that I eat in a month;
3 = more than I typically eat in a month but less than double the amount;
4 = about double the amount I typically eat in a month;
5 = more than double the amount I typically eat in a month

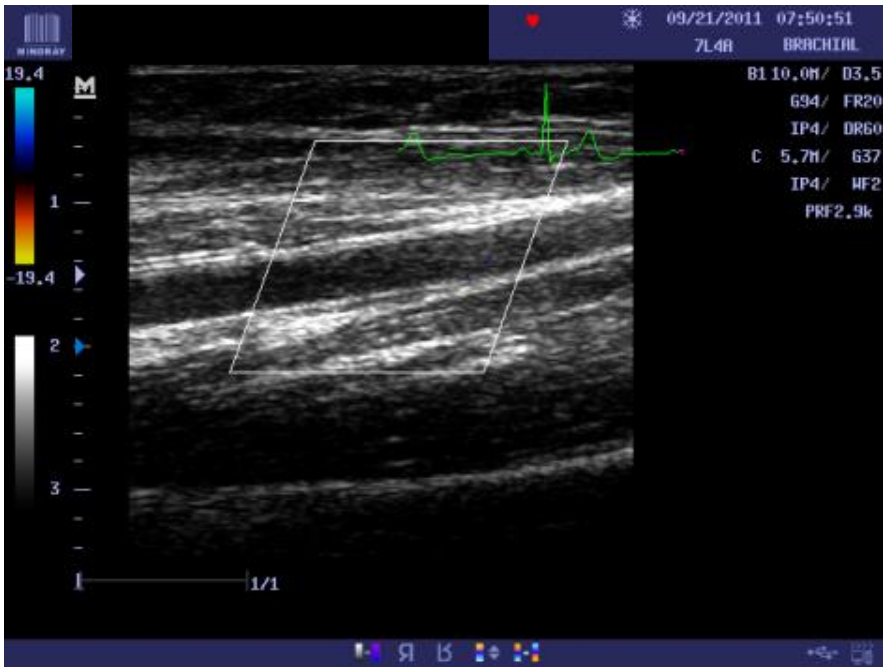
1)	In the last month how much shellfish (e.g., shrimp, crab, lobster, etc.) have you eaten	1	2	3	4	5
2)	In the last month how much fin-fish (e.g., tuna, salmon, swordfish, etc.) have you eaten	1	2	3	4	5
3)	In the last month how much poultry (e.g., turkey, chicken, ostrich, duck, quail, emu, etc.) have you eaten	1	2	3	4	5
4)	In the last month how much beef (e.g., sirloin, chuck, brisket, etc.) have you eaten	1	2	3	4	5
5)	In the last month how much beef (e.g., ground, flank, rib, etc.) have you eaten	1	2	3	4	5
6)	In the last month how much pork (e.g., cured ham, sirloin, tenderloin, etc.) have you eaten	1	2	3	4	5
7)	In the last month how much lamb have you eaten	1	2	3	4	5
8)	In the last month how much veal have you eaten	1	2	3	4	5
9)	In the last month how much game (e.g., buffalo, elk, moose, goat, rabbit, deer, antelope, boar, etc) have you eaten	1	2	3	4	5
10)	In the last month how much luncheon meat/sausage (e.g., turkey, chicken, roast beef, pork, etc.) have you eaten	1	2	3	4	5
11)	In the last month how much dairy (e.g., milk, cheese, yogurt, egg, etc.) have you eaten	1	2	3	4	5
12)	In the last month how much vegetables (e.g., spinach, carrots, tomato, etc.) have you eaten	1	2	3	4	5

13) In the last month how much nuts/seeds (e.g., sesame, pumpkin, sunflower, etc) have you eaten	1	2	3	4	5
14) In the last month how much soy protein/tofu have you eaten	1	2	3	4	5
15) In the last month how many gelatin desserts have you eaten	1	2	3	4	5

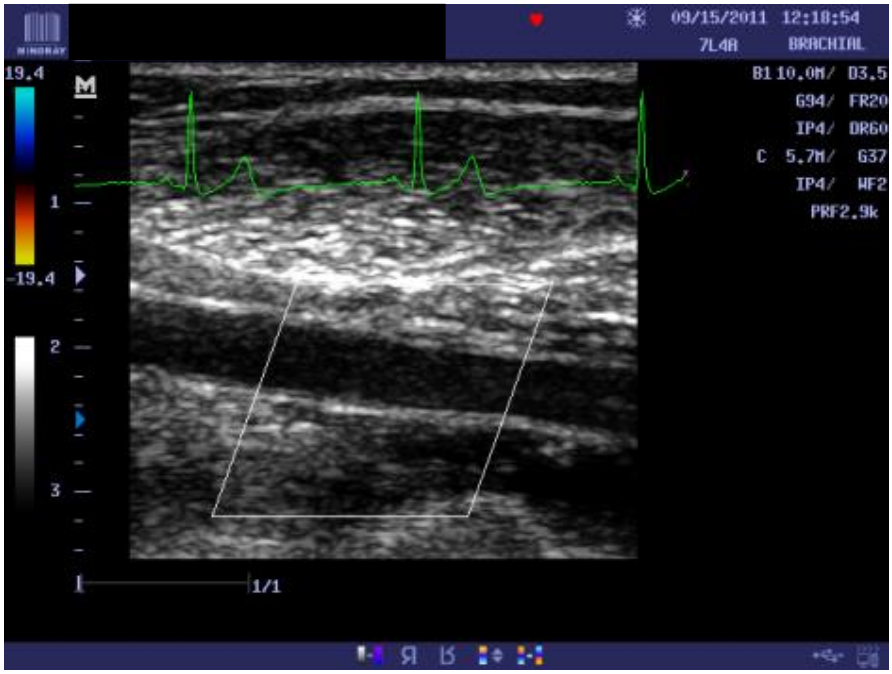
Appendix I



Carotid Image



Brachial Image



Popliteal Image

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