

ACUTE VASCULAR EFFECTS OF AQUATIC AND LAND TREADMILL
EXERCISE IN PRE-HYPERTENSIVE MEN

A Dissertation

by

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ABSTRACT

Chronic aquatic treadmill (ATM) exercise has previously been shown to offer similar benefits in cardiovascular health as traditional land treadmill (LTM) exercise with the added benefit of reduced blood pressure (BP) reactivity and increased skeletal muscle endothelial nitric oxide synthase content. The purpose of the present study was to determine the acute vascular effects of ATM and LTM exercise on resting, post-exercise, ambulatory BP, flow-mediated dilation (FMD), plasma nitrates/nitrites (NO), and carotid-femoral pulse wave velocity (PWV) in physically untrained, pre-hypertensive men. Following BP screening and a graded exercise test, 19 subjects completed the study (32 ± 12 years, 180 ± 7 cm, 91.9 ± 24.4 kg, 38.2 ± 8.4 ml·kg⁻¹·min⁻¹, 29.5 ± 9.9 % fat, $130/78 \pm 7/8$ mmHg). Subjects completed 2, 3-day acute exercise sequences, one for each exercise mode with 1-2 weeks between modes. Whether subjects began with ATM or LTM was randomized and counterbalanced. Each 3-day sequence included the following: day 1 exercise, day 2 exercise, and day 3 follow-up (3F). For each exercise day the following measurements were made: pre-exercise – BP, FMD, PWV, blood; immediately post-exercise (IPE) – blood; 1-hour post-exercise – blood, FMD, PWV. BP was measured following 10-minutes of seated rest prior to exercise and every 10 minutes from 20-60 minute post-exercise. Ambulatory BP was measured following each exercise session. Day 3F involved only BP, blood, FMD, and PWV measures. A 2-way repeated measures ANOVA was the primary model of statistical analysis. The specific analyses used for each dependent variable were as

follows: 1) resting BP – 2 (Mode: ATM vs. LTM) x 3 (Day: 1, 2, 3F); 2) post-exercise blood pressure change and ambulatory blood pressure – 2 (Mode) x 2 (Day); 3) FMD and PWV – 2 (Mode) x 5 (Time point: Day 1 Pre, Day 1 Post, Day 2 Pre, Day 2 Post, Day 3F); 4) change in FMD and PWV – 2 (Mode) x 3 (Time point: 1, 24, 48 hours post-exercise); 5) plasma nitrates/nitrites – 2 (Mode) x 7 (Time point: Day 1 Pre, 1 IPE, 1 1hr, Day 2 Pre, 2 IPE, 2 1hr, Day 3F); 6) plasma volume change – 2 (Mode) x 6 (Time point: Day 1 IPE, 1 1hr, Day 2 Pre, 2 IPE, 2 1hr, Day 3F). While there was a main effect for mode for resting diastolic BP (DBP) and mean arterial pressure (MAP) across the 3 days, these differences existed prior to the first exercise session. There were no differences in the reduction in resting systolic BP, DBP, and MAP (~2.5 mmHg) between modes across the 3 days. There was a main effect for day on resting DBP (Day 1: 74.2 mmHg, Day 2: 72.2 mmHg, Day 3F: 72.0 mmHg) and MAP (Day 1: 91.5 mmHg, Day 2: 90.3 mmHg, Day 3F: 89.5 mmHg), indicating a reduction in resting BP following the exercise sessions. Neither ambulatory BP (136/78 mmHg) nor post-exercise BP (~2 mmHg reduction in SBP) differed between mode or exercise day. There was a trend for a mode specific difference ($p = 0.076$) for a greater FMD response for ATM. FMD increased from ATM1 pre-exercise ($6.5 \pm 3.9\%$) to ATM1 1-hour post-exercise ($7.4 \pm 4.7\%$) and ATM1 24-hour post-exercise ($7.4 \pm 4.3\%$), although there were no differences in plasma nitrates/nitrites. Overall these results demonstrate that acute ATM exercise offers similar BP benefits as LTM exercise with the potential for enhanced FMD and improved endothelial function. These data support the efficacy of ATM as an exercise modality to benefit cardiovascular health and mitigate disease risk.

DEDICATION

To the Texas A&M Triathlon Club,
without whom I would have never survived my time here.

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NOMENCLATURE

| | |
|-----------------|-----------------------------------|
| ATM | Aquatic Treadmill |
| LTM | Land Treadmill |
| FMD | Flow-Mediated Dilation |
| PWV | Pulse Wave Velocity |
| NO | Nitric Oxide |
| eNOS | endothelial Nitric Oxide Synthase |
| BP | Blood Pressure |
| SBP | Systolic Blood Pressure |
| DBP | Diastolic Blood Pressure |
| MAP | Mean Arterial Pressure |
| AMBP | Ambulatory Blood Pressure |
| HR | Heart Rate |
| VO ₂ | Volume of Oxygen Consumption |

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CHAPTER I

INTRODUCTION

Blood pressure reactivity and ambulatory blood pressure are important markers of cardiovascular health, both of which can be influenced by exercise (85). A recent meta-analysis of 24-hour ambulatory blood pressure changes in response to endurance exercise training concluded average reductions in systolic blood pressure (SBP) of 3.2 mmHg and diastolic blood pressure (DBP) of 2.7 mmHg for daytime ambulatory pressures (15). In regard to acute blood pressure reactivity to exercise, exercise training on average reduces SBP by 7 mmHg (85).

Aquatic treadmill (ATM) training is a novel mode of aerobic exercise training that has been shown to offer similar cardiovascular benefits as traditional land treadmill (LTM) training (35) with the added benefit of a greater reduction in blood pressure reactivity following training (57). Unpublished data from our laboratory also indicate a tendency for a greater post-exercise SBP hypotensive response during 2 hours of recovery from an acute bout of ATM exercise in recreationally active college men (ATM: 113 ± 10 mmHg; LTM: 116 ± 8 mmHg; $p = 0.059$), similar to previous findings in healthy, untrained women (92). Given the evidence of ATM exercise having a greater impact on the acute, post exercise hypotensive response, as well as the chronic benefits of reduced blood pressure reactivity with ATM training, a comparison of 24-hour ambulatory blood pressures between aquatic and land treadmill exercise is warranted.

Vascular reactivity is an important component of blood pressure regulation. Endothelial nitric oxide synthase (eNOS) expression and activity stimulates NO production, and NO acts as a vasodilator on the vascular smooth muscle. Patients with coronary artery disease (CAD) show increased eNOS expression and activity following exercise training (38), as do exercised rodents (24, 101). While less is known regarding eNOS activity following training in humans outside the clinical setting, skeletal muscle eNOS content was higher in ATM trained subjects than LTM trained subjects following a 12-week aerobic exercise program (57). In addition to eNOS expression, there is evidence of increased NO availability following training in older humans (105). NO bioavailability is a marker of endothelial function and can be measured by the presence of NO metabolites or nitrites in the blood. A reduction in plasma nitrites and FMD was observed in patients with endothelial dysfunction relative to healthy controls (54). Four weeks of aerobic exercise training was shown to increase basal production of NO in hypercholesterolemic subjects (63) and healthy men (53). Acutely, plasma NO levels increased slightly after one exercise bout in cardiac patients, but increased to a much greater extent following a second day of exercise (126). Given the previously observed increases in skeletal muscle eNOS content in ATM trained subjects (57), the present study attempted to compare the acute production of NO through measurement of plasma nitrite levels following ATM and LTM exercise.

FMD is another important marker of endothelial function and predictor of CVD risk. Impaired FMD was also a significant predictor of an increased SBP response to exercise in Framingham Heart Study patients, even after adjusted for traditional

cardiovascular disease (CVD) risk factors and resting blood pressure (109). There have been varying results regarding the effects of an acute exercise bout on FMD post exercise. Much of this inconsistency is due to variation in the population studied, the mode, duration, and intensity of exercise, and the time that FMD is measured post exercise. In healthy males, cycling at higher intensities (70 and 85% max HR) for 30 minutes resulted in impaired FMD immediately post exercise, whereas there was no change in FMD at low intensities (50% max HR) even when baseline diameter and the FMD shear stress stimulus were controlled for post-exercise (7). In overweight men who were active, an acute bout of exercise, regardless of exercise intensity, augmented FMD one hour post exercise, whereas FMD was attenuated in the inactive subjects (40). The total exercise dosage or caloric expenditure is also a concern, as it has been shown in healthy, active men that acute exercise, regardless of intensity, in a total dosage equivalent to 30 minutes at 50% max resulted in improvements in FMD immediately post exercise and at one and two hours post. However, FMD either remained the same or was attenuated at both high and low intensities when total work and duration were increased (47). FMD was similar to pre-exercise levels at 1 hour, 24-hours, and 48-hours following exercise consisting of high intensity intervals (5 x 5 minutes at 90% max) in untrained subjects. Conversely, FMD was impaired at 1 hour in highly trained subjects under the same protocol, which was largely attributed to much larger baseline diameters at 1 hour post exercise (93). Despite the inconsistencies in these findings, the post exercise measurement of FMD is considered appropriate assuming proper considerations are made and guidelines followed (81). Given the ambiguity in the effect of various

exercise protocols on post-exercise FMD changes, further comparing the influence of exercise mode (ATM vs. LTM) in the same population could be of benefit, especially given the previous findings of increased eNOS content in muscle following ATM training (57).

As previously discussed, both blood pressure reactivity to exercise and ambulatory blood pressure are important measures of cardiovascular health. Furthermore, endothelial function is a key marker of vascular health, plays a role in blood pressure reactivity, and independently predicts CVD risk. Aquatic treadmill exercise has previously been shown to offer some unique cardiovascular adaptations. Therefore the purpose of this study was to determine the effects of an acute bout of both aquatic and land treadmill exercise on FMD, plasma nitrite levels (marker of nitric oxide production), and ambulatory blood pressure in pre-hypertensive, sedentary men.

CHAPTER II

BACKGROUND

Introduction

Cardiovascular disease (CVD), which is largely contributed to by arterial dysfunction, is the leading cause of morbidity and mortality. Vascular dysfunction occurs with aging and can be observed independent of other major CVD risk markers. For this reason, vascular health measures can be useful in determining CVD risk in otherwise healthy, aging populations. The most common measures of arterial health look at arterial stiffness of the large arteries, as well as endothelial function of the peripheral arteries. Seals and colleagues offer thorough reviews on these topics (97, 99). The different techniques for each of these measures will be discussed, along with their clinical significance in regards to other traditional CVD risk markers, particularly hypertension. Furthermore, the role of exercise in attenuating vascular aging will be explored with special concern for the physiological effects of aquatic exercise.

Endothelial Function

Background

Peripheral arterial function is most commonly measured through ultrasonography or strain-gauge plethysmography by observing endothelial dependent dilation (EDD) of the brachial artery in response to acetylcholine administration or flow-mediated dilation (FMD) induced by reactive hyperemia following forearm occlusion. Reviews by Seals (97, 99) focus on aging as the primary explanation for arterial dysfunction, even in the

absence of disease. Green and colleagues (33, 34) offer further insight, focusing on the differences between responses to training interventions with particular consideration given to nitric oxide (NO) mediated endothelial function in different populations (young, old, CVD). Special considerations in terms of training responses include: age, presence of disease, length of training intervention, mode of exercise (aerobic vs. strength training, localized vs. whole body), and intensity of exercise.

EDD is reduced in sedentary, middle-aged adults (40-50 years) without CVD (11). It has also been shown that in healthy, middle aged men, FMD correlates poorly with traditional CVD risk markers, despite the presence of impaired FMD measures (65). This indicates that in otherwise healthy individuals, impaired EDD may serve as an early indicator of cardiovascular problems. When CVD risk markers are present, impaired EDD is still commonly found. Hypertensive individuals, regardless of race, had impaired EDD (30). Additionally, elevated systolic blood pressure responses to exercise were correlated with impaired EDD, as well as increased arterial stiffness (109), indicating that in the presence of traditional CVD measures, arterial structural and functional measurements are associated. Cross sectional studies have shown that middle-aged men participating in regular aerobic activity do not demonstrate the same declines in EDD observed in age-matched sedentary men (20, 105).

Chronic Exercise Training

Short-term aerobic exercise interventions have shown that middle-aged men can improve EDD with as little as 3 months of moderate aerobic activity (20). However, more long-term aerobic interventions (24 weeks) among 50-75 year old men and women

did not show significant changes in EDD (9). While limited human training studies exist for such a prolonged duration, animal models have shown similar findings. Peripheral artery NO dependent vasodilation was not enhanced following 16-weeks of training in pigs (68) or rats (51). Therefore it appears that the length of the training intervention may play an important role in the observed changes in EDD. While complete mechanisms will be subsequently discussed, one hypothesis is that the NO dependent changes in response to shear stress with initial exercise are responsible for the augmented EDD observed with short term training (<12 weeks). However, more long term training interventions may result in reduced arterial shear stress due to more permanent enlargement and remodeling of the vessel (33).

Age and presence of disease appear to be important factors in training responses. There are consistent improvements observed in EDD in short term, aerobic training interventions for aged subjects, individuals with heart disease, and individuals with traditional CVD risk markers (33, 97, 99). However, young healthy subjects do not consistently show improvements in NO dependent endothelial function in response to exercise training (33). Training volume, training intensity, duration of training programs, and the methods employed to measure endothelial function are all important factors in distinguishing the responses observed in younger populations. Ten weeks of high-volume exercise including running (3 miles/day) and strength training (3 times/week), resulted in modest improvements (2.2-3.9%) in FMD in army recruits (13). However, aerobic exercise involving high intensities (1 hour running, 70-80% VO₂max, 4 days/week, 3 months) in active, young males actually resulted in a decline in endothelial

function, which was attributed to decreased levels of circulating antioxidants (5). Goto et al. (32) compared responses to cycling exercise (12 weeks, 5-7 days/week, 30 minutes/day) in healthy, young men separated into three groups based on training intensity (25%, 50%, 75% VO₂max). The moderate intensity (50% VO₂max) group significantly improved EDD, whereas no changes were observed in the low and high intensity groups. The high intensity group showed increased markers of oxidative stress (8-hydroxy-2'-deoxyguanosine and malondialdehyde-modified low-density lipoprotein), while the moderate intensity group tended to decrease these indices (32). The former investigators (13) showing improvements in EDD in young men with large training volume assessed endothelial function by ultrasound imaging of the brachial artery in response to FMD induced by reactive hyperemia. While the latter studies (5, 32) showing an attenuated effect of high training intensities on EDD assessed endothelial function via ACh administration and change in flow by strain-gauge plethysmography. While past research (83) has indicated that strain-gauge plethysmography and Doppler ultrasound do not correlate well, a more recent publication (44) indicated that these two methodologies are closely related. Nonetheless, the particular methodologies involved in assessing EDD should be an important consideration in reviewing contradictory findings. In addition to the effects of training volume (5) and training intensity (13) on functional endothelial changes in young subjects, the duration of the training program should also be a consideration, as discussed previously with older populations (9).

Acute Exercise Training

There have been varying results regarding the effects of an acute exercise bout on FMD post exercise. Much of this inconsistency is due to variation in the population studied, the mode, duration, and intensity of exercise, and the time that FMD is measured post exercise. In healthy males, cycling at higher intensities (70 and 85% max HR) for 30 minutes resulted in impaired FMD immediately post exercise, whereas there was no change in FMD at low intensities (50% max HR) even when baseline diameter and shear were controlled for post-exercise (7). In overweight men who were active, an acute bout of exercise, regardless of exercise intensity, augmented FMD one hour post exercise, whereas FMD was attenuated in the inactive subjects (40). The total exercise dosage or caloric expenditure is also a concern, as it has been shown in healthy, active men that acute exercise, regardless of intensity, in a total dosage equivalent to 30 minutes at 50% max resulted in improvements in FMD immediately post exercise and at one and two hours post. However, FMD either remained the same or was attenuated at both high and low intensities when total work and duration were increased (47). FMD was similar to pre-exercise levels at 1 hour, 24-hours, and 48-hours post exercise consisting of high intensity intervals (5 x 5 minutes at 90% max) in untrained subjects. However, FMD was impaired at 1 hour in highly trained subjects under the same protocol, which was largely attributed to much larger baseline diameters at 1 hour post exercise (93). This reduction in FMD occurred despite enhanced NO bioavailability and antioxidant status, emphasizing the importance of baseline diameter in the FMD response since a larger vessel will experience less shear for a given flow stimulus. A

review of the acute exercise model to assess FMD responses concludes it to be acceptable with the following considerations: serial FMD measures do no effect subsequent outcomes, the reproducibility of FMD post exercise is acceptable, dilation of an artery post-exercise may reduce FMD response, altered sympathetic nervous system activity post exercise may play a role in the FMD response, and finally the exact time point to best observe FMD changes post exercise remains unclear (81).

Omega-3 Fatty Acids

A meta-analysis of placebo controlled trials involving 0.45 to 4.5 g per day of omega-3 fatty acids over the median course of 56 days concluded there was a 2.3% improvement in FMD and no change in endothelial-independent dilation (120). While the exact mechanisms underlying these changes are unknown, proposed explanations include: increased membrane fluidity of endothelial cells due to omega-3 incorporation, reduction of inflammatory factors, and reduced platelet aggregation and vascular smooth muscle growth (120).

Blood Pressure Relationship

It has been observed that exertional hypertension experienced with a single bout of weight lifting may have an acute impairment on endothelial function in sedentary subjects immediately following the exercise bout (49, 87). However, in subjects who were experienced with either strength training or aerobic training, a single bout of strength training actually improved FMD (87). Furthermore, individuals that cross-trained regularly did not show any further protective benefit compared to the strength trained and aerobic trained groups (87). These are important considerations when

considering the acute impact of a resistance exercise bout and exertional hypertension on endothelial function.

Mechanisms

Mechanisms for reduced EDD with aging and disease include reduced NO availability, increased oxidative stress, and increased vasoconstrictor molecules; all of which may potentially be reversed with aerobic activity (97, 99). The differences in endothelial function observed between sedentary, older adults and their younger counterparts are largely explained by decreased nitric oxide availability (105). Endothelial nitric oxide synthase (eNOS) expression and activity stimulates NO production, and NO acts as a vasodilator on the vascular smooth muscle. Patients with coronary artery disease (CAD) show increased eNOS expression and activity following exercise training (38), as do exercised rodents (24, 101). Less is known regarding eNOS activity following training in humans outside the clinical setting, but there is still evidence of increase NO availability following training in older humans (105). While NO is a potent vasodilator, alterations in vasoconstrictor levels with aging, might also explain impaired EDD. Endothelin-1 (ET-1), an endothelial produced vasoconstrictor, levels are higher in sedentary, older men (23).

Other than reduced eNOS expression, increased oxidative stress is another reason that NO availability is reduced in sedentary adults (97, 99). Improved EDD is observed in both elderly rodents (24) and humans (105) when antioxidants were administered. This improvement in EDD is not observed in younger, active populations, indicating preserved antioxidant function in these groups. CAD patients showed decreased levels of

reactive oxygen species and NADPH oxidase in response to exercise training (2), and NADPH oxidase inhibition in mice increased NO availability (24). Furthermore, expression of the antioxidant enzyme superoxide dismutase (SOD) is increased in the arteries of exercised rodents (24, 114), and administration of an SOD type agent restored NO dependent EDD in sedentary mice (24). NO production by eNOS is also dependent on the co-factor tetrahydrobiopterin (BH_4), which is oxidized to the inactive form, BH_2 . Reduced levels of BH_4 were observed in sedentary, aged humans with NO dependent endothelial dysfunction (41). Administration of BH_4 improved EDD in sedentary, aged adults, but not young or exercise trained older adults (25). Together, these findings suggest that increased oxidative stress in sedentary, aged individuals impairs endothelial function, at least in part through decreasing NO bioavailability.

Whyte and Laughlin (123) provide a thorough mechanistic review of the changes that occur in the vasculature in response to aerobic exercise and training with particular concern for the endothelial cells response to shear stress experienced with increased blood flow. Increased longitudinal blood flow through an artery causes shear stress to the endothelial cells, which causes an increase in NO release due in part to the phosphorylation of eNOS by AKT. NO is produced by eNOS in the conversion of arginine to citrulline. NO then diffuses from the endothelium to the vascular smooth muscle where it causes vasodilation through a cyclic guanosine monophosphate (cGMP) mechanism that hyperpolarizes the cell. Long term training can help to chronically upregulate anti-atherogenic genes (56) and more favorably align the endothelial cells (123), which help to preserve NO availability and also aid in vascular remodeling.

Mechanotransduction is necessary to translate the shear stress signal applied to the endothelium to the actual biochemical response. While the exact mechanotransduction mechanisms are not completely understood, Hahn and Schwartz (37) review a number of proposed factors: cytoskeleton (microtubules, actin, intermediate filaments), adhesion receptors (PECAM1), luminal membrane proteins (heterotrimeric G proteins, ion channels, endothelial glycocalyx), and primary cilium.

Shear stress is essential to mediating the endothelial adaptations to exercise. During bilateral handgrip training with one arm cuffed to decrease shear rates, only the non-cuffed arm experienced improvements in FMD (110). In vitro studies have also revealed endothelial cells exposed to hydrostatic pressure alone without flow showed decreased eNOS mRNA expression, whereas increased shear stress from simulated flow increased eNOS expression, indicating that the shear stimulus is essential (67). Increased hydrostatic pressure simulated by passive arm hanging had a negative impact on subsequent brachial FMD measures, but sitting versus laying supine did not have the same negative consequence on popliteal artery FMD, indicating an arterial specific response to increased pressure and endothelial function (82). It is important to note that in both of these examples, increased hydrostatic pressure is occurring in the absence of substantial increases in shear rates that would be experienced with increased blood flow with activity. Some level of pressure is necessary for endothelial cell proliferation as in vitro endothelial cells increased in cell number to the greatest degree when exposed to biological pressures (120 mmHg) compared to lower pressures and down to atmospheric levels (103). Rather than only considering mean pressure, the pulse pressure can also

affect vessel dynamics. Isolated rat aorta exposed to a constant flow but varying pulse pressure amplitudes experienced lower mean pressures, increased distension, and likely a reduced shear stress stimulus (42). However given that flow was experimentally held constant, this relationship between pulse pressure and shear may not hold true in vivo with variable flow.

Plasma Nitrite Measurement

NO bioavailability is a marker of endothelial function and NO byproducts can be measured in the plasma. For instance, plasma nitrite levels were previously shown to mirror eNOS activity (59). Furthermore, a reduction in plasma nitrites and FMD were observed in patients with endothelial dysfunction relative to healthy controls (54). Four weeks of aerobic exercise training was shown to increase basal production of NO in hypercholesterolemic subjects (63) and healthy men (53). Plasma NO levels increased slightly after one exercise bout in cardiac patients, but increased to a much greater extent following a second day of exercise (126). NO metabolites can be measured via the Griess reaction to quantify nitrite after nitrates are reduced (115). Exhaled NO has been found to more closely reflect ventilatory rates rather than blood flow during exercise (86), likely making plasma NO a better indicator of vascular activity.

FMD Measurement

Assessment of FMD via ultrasonography is a non-invasive method for assessment of endothelial function. The brachial artery can be imaged longitudinally before and after forearm occlusion to measure the response to increased flow. Procedural

guidelines for these methods have been established by the International Brachial Artery Reactivity Task Force (18) and are further outlined in the methods section.

Arterial Stiffness

Background

In addition to impaired endothelial function seen with sedentary aging, increased stiffness of large arteries is also a risk factor for CVD (72, 99, 119). Large arteries should have a certain degree of elasticity and compliance that allows for expansion during cardiac systole, followed by recoil during diastole to continually propagate blood to downstream tissues. Stiffening of the large arteries can result in several negative effects: increased cardiac afterload, impaired coronary blood flow, adverse arterial remodeling, and end-organ damage (113). Increases in arterial stiffness may be observed in subjects with otherwise low cardiovascular risk scores and prior to the development of traditional risk factors (65). However as cardiovascular disease progresses, subjects with the greatest number of risk factors have been shown to have the greatest degree of aortic stiffening (62).

While there are various measures of assessing arterial stiffness, carotid-femoral pulse wave velocity (PWV) is considered the gold-standard for determining central arterial stiffness (61, 113, 117) and has the greatest predictive value for CVD risk relative to other methods (72). PWV has been found to be a significant predictor of cardiovascular risk in both healthy and hypertensive subjects (8, 43, 60, 113, 124). Reviews by Seals and colleagues have concluded that there is an increase in arterial stiffness observed with aging, but this is attenuated in active, older adults and can be

restored with exercise interventions in previously sedentary groups (97, 99). However, this may not be the case in individuals with long-term hypertension (26, 98). This review will determine the effects of exercise on arterial stiffness dependent on the population. It will further investigate the association between arterial stiffness and blood pressure. Finally, it will explore the mechanisms behind changes in arterial stiffness, as well as the techniques necessary for assessment.

Exercise Training

The benefits of exercise on arterial stiffness have been consistently demonstrated in cross sectional studies of older adults who demonstrate enhanced arterial health relative to sedentary peers (77, 99, 106, 107, 116). While there is a smaller body of evidence concerning exercise training interventions aimed to improve or restore arterial stiffness measures in middle-aged and older individuals, it appears that aerobic activity can enhance arterial health in most populations (74, 107). Central arterial compliance was improved to the level of endurance trained peers in previously sedentary, middle aged and older men following 3 months of aerobic walking exercise (107). These changes occurred independently of body composition and blood pressure (107). Additionally, post-menopausal women who were already on hormone replacement therapy were able to restore carotid arterial compliance following 3 months of brisk walking (74).

Seals et al. (98) found that in post-menopausal women dietary sodium restriction had a greater impact on lowering resting SBP than a walking exercise program over the course of 3-months. Furthermore, only the sodium restricted group decreased 24-hour

blood pressures and PWV in the trial. Since the exercising group failed to improve markers of arterial stiffness, it is important to consider the exercise prescription. Subjects exercised on their own from a recommended range of 30-45 minutes/day for 3-4 days/week at 40-50% to 65-80% of maximal heart rate as the 3 months progressed. They report measuring compliance with heart rate monitors, but no data was provided as far as the average exercise frequency, intensities, or durations for the exercise group. The sodium restricted group was asked to keep sodium intake below 2.4 g per day.

Given the limited data on training interventions, it is not currently known what the required frequency, intensity, or duration of aerobic exercise that is necessary to elicit positive changes in arterial stiffness (99). Additionally, short-term aerobic training (8-weeks moderate intensity cycling) did not improve arterial stiffness in patients with isolated systolic hypertension, indicating that the population studied is also important to the exercise intervention (26). While aerobic exercise training has been shown to decrease arterial stiffness, a recent meta-analysis concluded that resistance exercise is actually associated with increased arterial stiffness in young subjects, although it is undetermined how this may affect cardiovascular risk in this population (73). In conclusion, it appears that aerobic exercise improves arterial mechanical properties in otherwise healthy, sedentary aged populations. And while there is no clearly established aerobic exercise prescription in order to do so, certainly the mode of exercise and hypertensive status of subjects should be considered.

Omega-3 Fatty Acids

A review of nutrient intervention studies concerning arterial stiffness concluded that omega-3 fatty acid supplementation was an effective means of reducing arterial stiffness (84). In obese Japanese men with metabolic syndrome, 1.8 g per day of eicosapentaenoic acid (EPA) for 3 months reduced brachial-ankle PWV independent of changes in blood pressure (94). The decreased arterial stiffness was in association with a reduction serum amyloid A-low-density lipoprotein, a novel oxidized LDL complex associated with vascular inflammation and arterial plaque activity (94). Additionally, increases in brachial-ankle PWV and arterial stiffness were attenuated over the course of one year of 1.8 g per day of EPA supplementation in older men and women with dyslipidemia (112). Finally, the same EPA dose over the course of 2 years reduced brachial-ankle PWV in type 2 diabetics (71).

Blood Pressure Relationship

A review by O'Rourke and Hashimoto concluded that central arterial stiffness was not modifiable independent of changes in blood pressure, although these findings were concluded largely from pharmaceutical interventions (80). And as previously discussed, Tanaka et al. (107) demonstrated improvements in arterial stiffness independent of body composition or blood pressure changes following a 3-month aerobic exercise intervention.

Carotid-femoral PWV was an independent determinant of the longitudinal rise in systolic blood pressure over the course of 5 years in both normotensive and untreated hypertensive subjects. Additionally in the normotensive subjects, PWV was an

independent predictor of future hypertension (75). Additionally, brachial-ankle PWV was found to be a significant, but weak predictor of SBP increases over time in middle-aged Japan men (111).

Mechanisms

Given the nature of studying large arteries in humans, there is little direct evidence explaining the mechanisms behind increased arterial stiffening with aging or its attenuation with physical activity (99). Proposed structural changes in the extracellular matrix included an increase in collagen and breakdown of elastin affecting the media, and possibly adventitia vascular layers as well. Increased inflammation and oxidative stress may also contribute to these changes, and endothelial dysfunction further contributes to vascular inflammation (113). In untreated hypertensive subjects, PWV was significantly related to plasma high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α). Furthermore, hs-CRP was an independent predictor of PWV in these hypertensive subjects (66). The relationship between hs-CRP and arterial stiffness has also been demonstrated in apparently healthy subjects (125). However, hs-CRP was not predictive of increases in SBP over time in a sample of middle-aged Japanese men (111).

Infusion of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) caused a decrease in PWV in sheep hindlimb (95). When healthy subjects exercised with saline induced plasma expansion, cardiac output, central venous pressures, and end diastolic volume all increased and systemic vascular resistance decreased, similarly to what has been observed with aquatic exercise. The increased cardiac preload due to

plasma expansion also increased levels of atrial natriuretic factor and guanosine 3',5'-cyclic monophosphate relative to controls at lower levels of exercise intensity, but were similar at higher exercise intensities likely due to the fact that heart rate and sympathetic stimulation had a greater effect (50).

Techniques

When the heart contracts, the left ventricle generates a pulse wave that travels throughout the systemic circulation. To determine carotid-femoral PWV, a device must be able to determine the time delay between the feet of the pulse waves from the common carotid artery to the femoral artery. This time difference divided by the distance between the two arterial sites is calculated as the PWV. Stiffer arteries will propagate a pulse wave more rapidly, resulting in a higher PWV measurement. As previously stated, of the various methods to determine arterial stiffness, carotid femoral PWV is considered the gold standard and has the greatest predictive value in determining disease risk (72). While pulse wave velocity can be determined over any two segments of the arterial tree, it is the stiffness of the central arteries that is a concern with aging, inactivity, and disease. Therefore, measures of peripheral arterial stiffness, indexed by measures such as brachial-ankle or carotid-radial pulse wave velocities, are not as appropriate as aortic pulse wave velocity, which is best assessed by the carotid-femoral measurement. Van Bortel and colleagues (117) have established key guidelines in measurement and calculation of carotid-femoral PWV in their expert consensus document and reference values for arterial stiffness have also been established (1). Complete details are outlined in the methods section.

Aquatic Treadmill Exercise

Resting Water Immersion

Immersion in water elicits certain cardiovascular changes even during resting conditions. Water exerts a hydrostatic pressure of 1 mmHg/1.36 cm (0.54 in) of water depth. Because of this, just 4 feet of immersion depth creates a force equivalent to nearly 90 mmHg (4). This increased hydrostatic force pushes blood up through the venous circulation, increasing central blood volume and cardiac filling, which results in lower resting heart rate, greater stroke volume, increased cardiac output and greater pulse pressures (28, 29, 36, 91). Also during resting water immersion to the neck, decreased sympathetic vasoconstriction and systemic vascular resistance have been observed at thermoneutral temperatures, resulting in lower diastolic pressures (3). Systolic pressures were unchanged during resting water immersion at various temperatures (33°C - 39°C, 91.4°F - 101.2°F) (122). At 33°C stroke volume and cardiac output increased by approximately 50% and 30%, respectively, despite a 10% reduction in heart rate. A 30% reduction in peripheral resistance resulted in a mean decrease of diastolic pressure of nearly 10 mmHg, which contributed to a 10% reduction in mean arterial pressure despite maintenance of systolic pressure. With the exception of elevated heart rates at higher temperatures, all of these cardiovascular changes were further magnified with increasing water temperatures (122).

Active Water Immersion

Elevated cardiac outputs and stroke volumes observed during resting water immersion were also recorded during upright aquatic cycling exercise relative to land

cycling during incremental exercise to exhaustion. Heart rates were only lower at higher exercise intensities in water, and systolic blood pressure was similar for a given intensity with both modes (12). The authors hypothesized that systolic pressures may have appeared the same because of the noninvasive form of measurement utilized or the activation of baroreceptors inducing reduction in heart rate, which was observed at least at higher exercise intensities for the aquatic cycling. Reduction in heart rate at higher exercise intensities might also be explained by reduced sympathetic nervous system activity, as indicated by lower norepinephrine levels during aquatic cycling at high intensities relative to land cycling in another investigation (14). Additionally, it is theorized that the cutaneous vascular beds may accommodate the increased cardiac output during aquatic exercise, although cutaneous blood flow was not directly measured (12). In a study of older adults walking at incremental treadmill speeds (2-3 miles/hour) on both a land and aquatic treadmills, physiological workload by measure of oxygen consumption, heart rate, perceived exertion, and systolic blood pressure were all higher for a given speed on the aquatic treadmill (22). Due to the fact that a given systolic blood pressure measurement was matched by treadmill speed between the two modalities and not VO₂ or HR, it is not appropriate to assume from this study alone that aquatic treadmill exercise at a truly matched physiological workload elicits greater systolic blood pressures than land treadmill exercise. When middle aged subjects performed incremental exercise on a land cycle ergometer and an aquatic cycle submerged (31°C) to the shoulders at matched levels of oxygen consumption (40, 60, and 80 percent VO₂max), cardiac output was greater at 40 and 80 percent VO₂max for the aquatic

cycle, and stroke volume was elevated at all stages for the aquatic cycle mode (100). Additionally, heart rate was lower at the highest intensity exercise stage for aquatic cycling. While systolic blood pressure was not significantly different statistically between modes for each exercise intensity, the aquatic cycling did elicit at SBP of 174 ± 22 mmHg compared to 164 ± 25 for land cycling at the 80 percent VO₂max stage. During the lower intensity exercise stages SBP did not differ by more than 5 mmHg on average between modes, so a greater difference appeared to occur at higher exercise intensities. Differences in diastolic blood pressure actually reached statistical significance at 80 percent VO₂max between modes (82 ± 10 vs. 60 ± 26 for aquatic and land, respectively). Additionally, diastolic pressure was essentially maintained at approximately 80 mmHg throughout the aquatic cycling stages, whereas the land based cycling resulted in a traditional drop in diastolic pressure from rest and throughout graded exercise. It appears that the greatest differences in cardiovascular measures come at higher exercise intensities between aquatic and land based exercise. Finally, while the acute effects of aquatic exercise on blood pressure has been explored, there are no long term studies determining the effects of chronic aquatic exercise training on exercise blood pressure.

Connelly and colleagues (14) found reduced norepinephrine and epinephrine at higher exercise intensities for similar graded, aquatic cycling exercise relative to land cycling. The reduced sympathetic stimulation at higher exercise intensities may also explain the reduced heart rates observed for aquatic exercise. Increase in central blood volume would stimulate the low-pressure baroreceptors, and increases in stroke volume,

systolic pressure, and pulse pressure should result in stimulation of arterial baroreceptors. Both of these mechanisms might explain the reduction in norepinephrine (90). The authors did warn that since venous measures of plasma norepinephrine were measured it is possible that higher exercise intensities did not result in reduced catecholamine production, but a greater clearance, which may be supported by higher cardiac outputs previously observed with aquatic exercise. In regards to the systolic blood pressure response to aquatic cycling, these researchers (14) did not find any consistent differences in systolic blood pressure in their younger subject pool (22-36 years old) compared to those previously discussed (100) in older, middle-aged subjects (38-61 years old).

Blood Pressure

Resting Blood Pressure

It is well documented that resting blood pressure can be reduced with exercise training. In normal, healthy subjects 4 weeks of treadmill exercise for 3 sessions per week, 30 minutes per session at 65-70% max was more effective than 60 minutes at 50% max for 5 days and 15 minutes at 80-90% max for 3 days at reducing resting blood pressure (52). Resting blood pressure has been shown to reduce significantly in as little as 3 training bouts without further reduction over the course of 12 weeks of training in normal, healthy subjects (69). Once training was ceased resting blood pressure returned back to pre-training levels in approximately two weeks (69). A recent, comprehensive meta-analysis on the effects of exercise on resting blood pressure concluded from endurance exercise studies lasting at least 4 weeks in duration that there was an average

reduction in SBP of 3.5 mmHg and DBP of 2.5 mmHg. Effect sizes were greatest in hypertensive subjects, men, moderate to high exercise intensities, exercise session durations of 30 to 45 minutes, total weekly exercise time less than 210 minutes, and interventions lasting less than 12 weeks. Age and frequency of training sessions per week had less of an impact on resting blood pressure changes (16).

Exercise Blood Pressure

Exercise blood pressure is considered an important marker of CVD risk. As previously discussed, increased carotid-femoral pulse wave velocity and impaired flow-mediated dilation were significant correlates of an increased systolic blood pressure response to exercise in Framingham Heart Study patients even after adjusted for traditional CVD risk factors and resting blood pressure (109). Aerobic exercise training is known to reduce the blood pressure response to exercise in a variety of populations (17, 104). In regard to exercise dosage, treadmill and cycle exercise of anywhere from 4 to 12 kcal/kg per week for 6 months were effective at reducing exercise systolic blood pressures in overweight, postmenopausal women. However, only the highest quantity of exercise expenditure significantly reduced diastolic pressures during exercise. These changes occurred despite no changes in resting blood pressures (104). Additionally, both low intensity (33% heart rate reserve) and high intensity (66% heart rate reserve) aerobic exercise training over 10-weeks were effective at reducing exercising systolic blood pressures in previously sedentary men and women over 55 years of age (17). Thus, it appears that a variety of aerobic exercise training prescriptions are effective at reducing exercise systolic blood pressures. During exercise in non-hypertensive, Asian subjects

(age 50 ± 16 years), there was a greater increase in angiotensin II levels in the group that showed an exaggerated blood pressure response (peak SBP 60 mmHg greater than baseline), whereas the increases in renin, aldosterone, and catecholamines were similar in both the normal and exaggerated exercise blood pressure groups. Therefore, the authors concluded that of the complex neurohormonal systems at play in regulating blood pressure, angiotensin II appears to have the greatest effect on the blood pressure response to exercise.

Ambulatory Blood Pressure

Ambulatory blood pressure measured over a 24-hour period can provide valuable information beyond that of traditional clinical resting blood pressure monitoring and may be a stronger predictor of CVD morbidity and mortality (78, 102, 118). A recent meta-analysis of 24-hour ambulatory blood pressure changes in response to endurance exercise training concluded average reductions in SBP of 3.2 mmHg and DBP of 2.7 mmHg for daytime ambulatory pressures. These reductions were similar between normotensive and hypertensive subjects, men and women, and automated blood pressure devices used (15). Three intermittent, 10-minute exercise bouts were more effective than one, 30-minute session of walking at reducing ambulatory systolic blood pressures in pre-hypertensive men and women (6). In terms of exercise intensity, 30-minutes of activity at 75% VO_2max had a longer lasting effect on reducing ambulatory blood pressure than at 50% max in hypertensive subjects (89). However these studies did not include comparison between modes of exercise. Given our lab findings of reduced blood pressure response to land exercise following aquatic treadmill exercise (57), a

comparison of ambulatory blood pressures compared between aquatic and land treadmill exercise is warranted. The European Society of Hypertension (78) and the British Hypertension Society (79) recommend specific guidelines concerning the measurement of ambulatory blood pressure, and details are outlined in the methods section.

Conclusion

Arterial stiffness and endothelial function are key markers of arterial health that can predict cardiovascular disease risk even prior to the presentation of traditional risk markers. Impairment in these arterial health measures are most pronounced in healthy, but sedentary older populations. Exercise can attenuate the deterioration in arterial health that is typically observed with aging. Additionally, both arterial stiffness and endothelial function have been predictive of future hypertension or elevated exercising blood pressures. An exaggerated systolic blood pressure response to exercise is also a concern for cardiovascular health. While traditional aerobic exercise seems to play a role in reducing the blood pressure response to exercise in older, sedentary subjects, less is known regarding different modes of aerobic exercise, particularly with concern to aquatic exercise. Aerobic exercise when submerged in water presents unique alterations to cardiovascular dynamics, as central venous pressure increases, stroke volume and cardiac output increase, heart rate decreases, and systolic pressures may increase in response to the increased cardiac output at higher exercise intensities. While there is still need for clarification in terms of the acute hemodynamic changes observed with aquatic exercise, even less is known of the chronic effects of aquatic exercise training. If unique

adaptations in arterial health or exercise blood pressure responses are observed with chronic aquatic exercise, there would be significant health implications.

Hypothesis and Specific Aims

- *Hypothesis:* This study sought to test the hypothesis that compared to traditional land treadmill exercise, an acute bout of aquatic treadmill exercise elicits a more favorable post-exercise vascular response in flow mediated dilation, pulse wave velocity, plasma nitrite levels, and resting and ambulatory blood pressure in sedentary, pre-hypertensive men.
- *Aim 1:* Determine the acute effects of ATM and LTM exercise on pre and post-exercise blood pressure and 24-hour ambulatory blood pressure
- *Aim 2:* Determine the acute effects of ATM and LTM exercise on FMD
- *Aim 3:* Determine the acute effects of ATM and LTM exercise on plasma nitrates/nitrites
- *Aim 4:* Determine the acute effects of ATM and LTM exercise on PWV
- *Rationale:* Our previous laboratory findings have shown that aquatic treadmill training reduces blood pressure reactivity and increases skeletal muscle eNOS content. The current research study will determine the acute effects of aquatic treadmill training on blood pressure and key markers of vascular health. If aquatic treadmill exercise elicits a more favorable vascular and blood pressure response than traditional, land treadmill exercise, this would have a significant impact on the prescription of aquatic treadmill exercise in the maintenance of vascular health and regulation of blood pressure.

CHAPTER III

METHODS

General Procedures

Sedentary, pre-hypertensive (SBP: 121-139 mmHg and/or DBP: 81-89 mmHg) men between the ages of 18-65 who were not currently taking anti-hypertensive medication were recruited from the Texas A&M University community to participate in the study. A total of 19 subjects completed all study requirements. Subject demographic information is listed in Table 3.1. There were 12 screen failures due to not meeting blood pressure screening criteria or abnormal electrocardiogram (ECG) and 4 dropouts due to time commitment. All procedures were approved by the Institutional Review Board for Human Subjects Research prior to subject recruitment and informed consent was obtained prior to subject participation.

| Variable | Average | SD | Range |
|--------------------|---------|------|--------------|
| Age (Years) | 32 | 12 | 19 - 59 |
| Height (cm) | 180 | 7 | 167 - 193 |
| Mass (kg) | 91.9 | 24.4 | 64.1 - 169.1 |
| VO2max (ml/kg/min) | 38.2 | 8.4 | 23.3 - 53.6 |
| Body Fat (%) | 29.5 | 9.9 | 8.0 - 44.7 |
| SBP (mmHg) | 130 | 7 | 121 - 142 |
| DBP (mmHg) | 78 | 8 | 66 - 90 |
| MAP (mmHg) | 96 | 6 | 83 - 108 |
| HR (bpm) | 75 | 15 | 53 - 109 |

Table 3.1. Subject demographics

Following an initial blood pressure screening and orientation visit, subjects reported to the lab on 7 separate occasions over the course of approximately 17-days. A sample study timeline is provided in Figure 3.1.

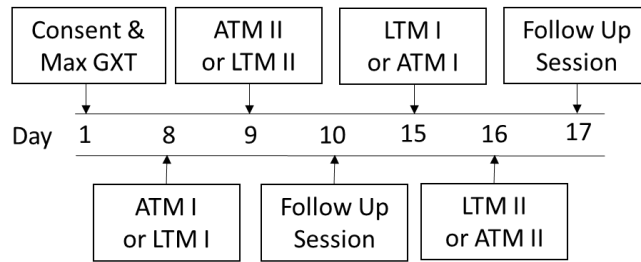


Figure 3.1. Study timeline

After screening, orientation, and consent, subjects completed a maximal graded exercise test (GXT) following the Bruce treadmill protocol with measurement of oxygen consumption (VO_2), ECG, blood pressure, and perceived exertion, as previously described (35). Approximately 7-days later, subjects then completed their first acute exercise session assigned to ATM or LTM in a randomized fashion. Following the first acute exercise session, subjects returned the following day for a second training session on the same mode as the previous day. Subjects then returned on the third day for follow-up measures only. This 3 day sequence was repeated 1-2 weeks later for the other exercise mode. Both the day of the week and time of day were matched for a given subject between modes. All acute exercise sessions included the following pre-exercise measures: blood pressure following 10 minutes of seated rest, blood draw following 15 minutes of seated rest, and FMD and PWV following 10 minutes of supine rest. Subjects exercised at ~60% of maximal VO_2 for a duration required to expend 300 kcal, as similar

volumes and intensities have been shown to augment FMD post exercise (46). During exercise, oxygen consumption was recorded for at least 1 minute every 10 minutes to confirm intensity and to match intensity and energy expenditure between modes. Heart rate (HR) and rating of perceived exertion (RPE) were also recorded every 10 minutes during exercise. Blood samples were taken in the seated position in a quiet, environmentally controlled room immediately post-exercise and again following 1-hour of seated rest. Additionally, FMD and PWV were measured 1-hour post exercise. Blood pressure was taken automatically every 10 minutes during the 1-hour post-exercise recovery period. A summary of acute exercise session procedures is provided in Figure 3.2. These procedures were repeated for the second day's session for a given exercise mode. A third follow up day, 24-hours following the last exercise session, included only a blood draw, blood pressure, FMD, and PWV measurement. Ambulatory blood pressure was also measured during the 24-hour period following each exercise session. These procedures were repeated 1-2 weeks later on the same days of the week and time of the day for the alternate exercise mode.

| <u>Pre-Exercise</u> | <u>Exercise</u> | <u>Immediately Post-Exercise</u> | <u>1-Hour Post-Exercise</u> | <u>24-Hour Post-Exercise</u> |
|--|---|--|--|--|
| <ul style="list-style-type: none"> • Blood Draw • 10 min supine • BP • FMD | <ul style="list-style-type: none"> • 60% VO₂max; 300 kcal • HR & RPE every 5 min • VO₂ & BP every 10 min | <ul style="list-style-type: none"> • BP • Blood Draw | <ul style="list-style-type: none"> • 1 hour supine • Blood Draw • BP • FMD | <ul style="list-style-type: none"> • Blood Draw • 10 min supine • BP • FMD |

Figure 3.2. Acute exercise session protocol

Oxygen consumption and ECG measures were made using a metabolic stress testing system (MGC Diagnostics, MN). All blood samples were obtained from the antecubital vein using standard venipuncture procedures while the subject was seated. NO metabolites were analyzed via the Griess reaction to quantify nitrites after nitrates were reduced (115) using a commercially available nitrate/nitrite colorimetric assay kit (Cayman Chemical Company, MI). Plasma samples were analyzed by an outside lab for complete blood count to determine hemoglobin and hematocrit levels. Changes in plasma volume were then determined as previously described (21) and nitrate/nitrite metabolite concentrations were adjusted accordingly. FMD measures were made via ultrasound (Logiq P6, GE Healthcare, UK) following recommended guidelines (18) and analyzed using specialized wall tracking software (Brachial Analyzer, Medical Imaging Applications-LLC, IA). Additionally, PWV was measured via the same ultrasound system following recommended procedures (117). Ambulatory blood pressure was monitored over 24 hours following established guidelines (88) with a commercially available, validated device (Oscar 2, SunTech Medical, NC).

Subject Recruitment

Subjects were recruited through bulk campus email and mail, posted flyers, and targeted blood pressure screenings on the Texas A&M University campus. Interested subjects were initially asked their age, physical activity habits, resting blood pressure, and whether or not they were taking blood pressure medication. Only male subjects who met the age criteria (18-65), were not currently participating in any regular exercise

routine, reported potentially high or unknown blood pressure, and not currently taking blood pressure medication were brought in for further screening.

Blood Pressure Screening

Interested subjects who met the initial screening criteria will come to the Applied Exercise Science Laboratory for official blood pressure screening to determine if they met the pre-hypertensive criteria (SBP: 121-139 mmHg and/or DBP: 81-89 mmHg). Subjects were directed to abstain from exercise, caffeine, alcohol, and nicotine on the day of screening and to avoid eating within 2 hours prior to screening. Upon arrival, subjects were seated for 10 minutes prior to any blood pressure measurements being made. Following 10 minutes of seated rest, blood pressure and heart rate were taken every 3 minutes for a total of 3 measures using an automatic blood pressure device (Dinamap Pro 1000, GE Healthcare, UK) using a properly sized cuff placed on the left arm. The average of 3 blood pressure readings was used to determine if blood pressure fell within the pre-hypertensive range. These procedures were repeated a second time prior to the maximal exercise test to confirm pre-hypertensive status. Following screening, eligible subjects were given an overview of the complete study details along with the informed consent document and a health history questionnaire.

Baseline Fitness Assessment

Following screening, eligible subjects completed a maximal graded exercise test (GXT) following the Bruce treadmill protocol (10) with measurement of oxygen consumption (VO_2), ECG, blood pressure, and perceived exertion, as previously described (35). Maximal VO_2 determined from this test was used for prescribing

target exercise intensity in subsequent acute exercise training sessions. Two or more of following criteria were required for the maximal test to be considered valid: 1) maximal heart rate within 10 beats of age-predicted max; 2) rating of perceived exertion ≥ 18 ; 3) respiratory exchange ratio >1.1 at exhaustion; or 4) O_2 uptake plateau despite further increases in workload. In addition to the maximal GXT, body composition was be assessed via DEXA (Lunar Prodigy Advance, GE Healthcare, UK) for further demographic information.

Acute Exercise Sessions

Approximately one week later, the subject completed their first acute exercise session assigned to ATM or LTM in a randomized fashion. Following the first acute exercise session, subjects returned the following day for a second training session on the same mode as the previous day. Subjects then returned on the third day for follow-up measures only. This 3 day sequence was repeated approximately 1-2 weeks later for the other exercise mode. All acute exercise sessions included the following pre-exercise measures: blood pressure following 10 minutes of seated rest, blood draw following 15 minutes of seated rest, and FMD and PWV following 10 minutes of supine rest. Subjects exercised at $\sim 60\%$ of maximal VO_2 for a duration required to expend 300 kcal, as similar volumes and intensities have been shown to augment FMD post exercise (46). During exercise, oxygen consumption was recorded for at least 1 minute every 10 minutes to confirm intensity and to match intensity and energy expenditure between modes. Heart rate (HR) and rating of perceived exertion (RPE) was recorded every 5 minutes during exercise.

Resting Blood Pressure

Prior to any measurements being made on each acute exercise session day and immediately upon arriving at the lab, subjects were seated, resting for 10 minutes. During this time they were asked to confirm whether they had abstained from alcohol, caffeine, nicotine, and if they were at least 8 hours fasted. Following 10 minutes of seated rest, an automatic blood pressure device (Dinamap Pro 1000, GE Healthcare, UK) was used to measure blood pressure and heart rate using a properly sized cuff placed on the left arm. A total of 3 measurements were taken with 3 minutes between each measure. The first measurement was discarded and the second and third measurements were averaged to determine resting blood pressure for the day. Following each exercise session, subjects sat resting for 60 minutes. Blood pressure was measured every 10 minutes from 20-60 minutes post-exercise and averaged to determine the post-exercise blood pressure. The post-exercise hypotensive response was calculated for each session by subtracting the average pre-exercise blood pressure from the average post-exercise blood pressure for a given day. Resting blood pressure measurements pre and post-exercise were made on all 19 subjects who completed the acute exercise sessions.

Blood Samples

All blood samples were made following standard phlebotomy procedures with the subject in the seated resting position and following an 8-12 hour fasting period. Blood samples were taken prior to acute exercise sessions, immediately post exercise, 1-hour post exercise, and 24-hours post exercise. Blood samples were drawn without stasis from an antecubital vein into 10 ml and 4 ml Vacutainer EDTA tubes containing for

plasma collection. Samples from 10 ml tubes were placed in a centrifuge (VanGuard V6500, Hamilton Bell, NJ) at 3400 rpm for 15 minutes at 4°C. Aliquots of plasma were stored at -80°C for later analysis. Plasma nitrite levels were determined from stored aliquots via the Griess reaction (115) using a commercially available nitrate/nitrite colorimetric assay kit (Cayman Chemical Company, MI). Samples from 4 ml tubes were sent out to external lab (St. Joseph Medical) for complete blood count (CBC) analysis to determine hemoglobin and hematocrit levels. Changes in plasma volume were then determined as previously described (21) and nitrate/nitrite metabolite concentrations were adjusted accordingly. Due to missed blood draws at one or more of the 14 time points, there were 13 of the 19 subjects who had all samples measured.

Flow-Mediated Dilation

Assessment of FMD was made via ultrasonography (Logic P6, GE Healthcare, UK) following procedural guidelines established by the International Brachial Artery Reactivity Task Force (18). Subjects reported to the laboratory following an 8 to 12 hours fast. While lying supine on a table in a temperature controlled room, the subject extended their right arm to the side so that the brachial artery could be imaged by a high-frequency linear transducer (10-12 MHz). Landmarks, such as veins, were noted on the ultrasound image to help ensure a constant image location of the artery and distance from the antecubital space and imaging site were marked and recorded for subsequent measurements. A manual probe-holding device was also utilized to ensure consistent vessel imaging. Baseline imaging was obtained and recorded to DVD (DVO-1000MD, Sony) for 1-minute and baseline pulse wave recordings were made within the ultrasound

imaging system. A blood pressure cuff was placed around the forearm and inflated to 200 mmHg for 5 minutes of occlusion. Following 5 minutes of cuff inflation, the pressure was released and pulse wave recordings made at 15 seconds post-occlusion. Diameter recordings from 30-120 seconds post occlusion were recorded to DVD. All DVD diameter recordings were analyzed by special wall tracking software (Brachial Analyzer, Medical Imaging Applications-LLC, IA) by a technician blinded to the exercise mode being reviewed. Diameter measurements were made at the end of diastole. FMD procedures were made prior to acute exercise sessions and again 1-hour post exercise as indicated in general procedures. FMD measurements for all time points were made on all 19 subjects.

Pulse Wave Velocity

Carotid-femoral PWV was measured following established guidelines by Van Bortel and colleagues (117) in their expert consensus document. PWV measures were made via ultrasonography (Logic P6, GE Healthcare, UK) on the right carotid and femoral arteries following the FMD measurement. The carotid and femoral site measures were made separately. Time was recorded from the top of the R wave on the QRS complex to the onset of the sharp inflection of the pulse wave recording. Time measurements were averaged over 6 cardiac cycles. The difference in the time delay between carotid and femoral sites was divided by the measured distance to determine pulse wave velocity. Distance between the carotid and femoral sites was estimated based on body height using the following formula: $\text{Distance (cm)} = \text{Height(cm)}/4 + 7.28 \text{ cm}$ (121). PWV measures were made prior to exercise and 1-hour post exercise. Due to

technical issues with equipment, PWV was recorded accurately from 16 of the 19 subjects at all time points.

Ambulatory Blood Pressure

Ambulatory blood pressure was measured in the 24-hours following each of the 4 exercise sessions. Guidelines from The European Society of Hypertension (78) and the British Hypertension Society (79) concerning the measurement of ambulatory blood pressure were followed using a validated ambulatory blood pressure device (Oscar 2, SunTech Medical, NC) (31). Following patient education and proper cuff placement, the device was programmed to take measurements every 20 minutes during waking hours and every 60 minutes during sleeping hours for 24 hours following each exercise session. Measurements were made in the non-dominant arm, and the same cuff and device were used each time. Subjects were instructed to keep their arm steady and at heart level during measurements while continuing with normal activity between measures. Additionally, subjects were asked to record the activity/posture at time of measurements, the time they go to bed and wake up, and any other issues on a provided diary card. In terms of reference values, ambulatory pressures less than 135/85 mmHg during waking hours and less than 120/70 mmHg while asleep are generally considered normal (78). Due to the lack of a large enough cuff for one subject, AMBP was recorded during all waking hours following all exercise sessions in 18 of the 19 subjects.

Diet Analysis

Subjects completed a diet log and activity log (Appendix B) on the day prior and the two days of the acute exercise sessions for each mode. Subjects were asked to eat a similar diet for both exercise mode trials and to avoid caffeine, alcohol, nicotine, and any other drugs or supplements during this 3-day period. Subjects were at least 8 hours fasted prior to each day's acute session. Subjects were also told to record any light activity completed during each 3-day sequence and were asked to abstain from purposeful exercise outside of that prescribed during the study sessions. This was confirmed verbally prior to each morning's session and by dietary records. Dietary analysis was made via NutriBase (CyberSoft Inc., AZ) nutrition software by averaging data from each 3-day sequence. Adequate dietary records were obtained for all sessions from 13 of the 19 subjects.

Statistical Design

A 2-way (mode x day/time point) repeated measures ANOVA was the primary model of statistical analysis. The specific analysis used for each dependent variable were as follows: 1) resting blood pressure – 2 (Mode: ATM vs. LTM) x 3 (Day: 1, 2, 3F); 2) post-exercise blood pressure change and ambulatory blood pressure – 2 (Mode) x 2 (Day); 3) FMD and PWV – 2 (Mode) x 5 (Time point: 1 Pre, 1 Post, 2 Pre, 2 Post, 3F); 4) change in FMD and PWV – 2 (Mode) x 3 (Time point: 1, 24, 48 hours post-exercise); 5) plasma nitrates/nitrites – 2 (Mode) x 7 (Time point: 1 Pre, 1 IPE, 1 1hr, 2 Pre, 2 IPE, 2 1hr, 3F); 6) plasma volume change – 2 (Mode) x 6 (Time point: 1 IPE, 1 1hr, 2 Pre, 2

IPE, 2 1hr, 3F). Statistical analyses were performed in IBM SPSS Statistics 23 (IBM, New York).

CHAPTER IV

RESULTS

Resting Blood Pressure

Resting blood pressure measurements taken prior to exercise on day 1, 2, and on a third follow-up day for each exercise mode are displayed for SBP (Figure 4.1), DBP (Figure 4.2), and MAP (Figure 4.3). A 2 (mode) x 3 (day) repeated measures ANOVA revealed no significant main effects or interaction for SBP.

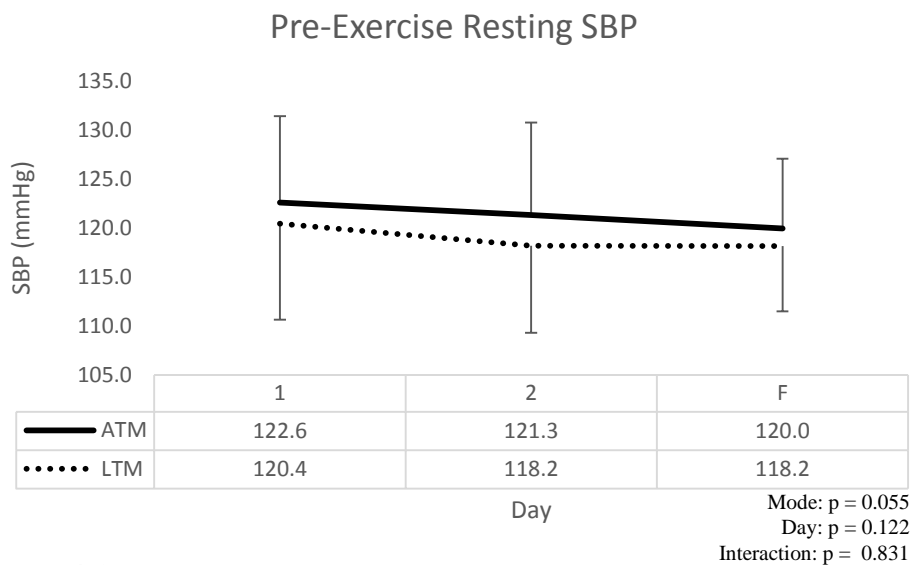


Figure 4.1. Resting systolic blood pressure (SBP) prior to land (LTM) and aquatic (ATM) treadmill exercise. Measured on exercise days 1 and 2, and a third follow-up (F) day. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. $N = 19$.

However, both mode and day were significant for DBP and MAP. Post-hoc (LSD) analysis showed diastolic pressures decreased from Day 1 (74 ± 8 mmHg) to Day

2 (72 ± 8 mmHg) and Day 3 (72 ± 7 mmHg). A dependent sample t-test showed diastolic pressure was higher prior to the first ATM session (75 ± 8 mmHg) than first LTM session (73 ± 9 mmHg). Despite the initial differences at baseline, diastolic pressure did decrease across days for each mode. It was reduced significantly from ATM1 (75 ± 8 mmHg) to ATM2 (73 ± 7 mmHg) and LTM1 (73 ± 9 mmHg) to LTMF (70 ± 7 mmHg). Similar to the initial differences observed at baseline, diastolic pressure was still higher for follow-up day 3 for ATM (74 ± 8 mmHg) compared to LTM (70 ± 7 mmHg). Post-hoc analysis for MAP showed Day 1 (91 ± 6 mmHg) was greater than Day 3 (90 ± 4 mmHg), and a t-test showed that LTM1 (90 ± 6 mmHg) was greater than LTMF (89 ± 4 mmHg). Overall, these results show that for DBP and MAP, there was a significant effect of exercise on the subsequent day's resting blood pressure, as blood pressure was generally lower on day 2 and the third follow-up day. While there was a significant main effect for exercise mode with higher pressure for ATM, this existed prior to the initial exercise session. There were no significant main effects or interaction for pre-exercise HR (Figure 4.4).

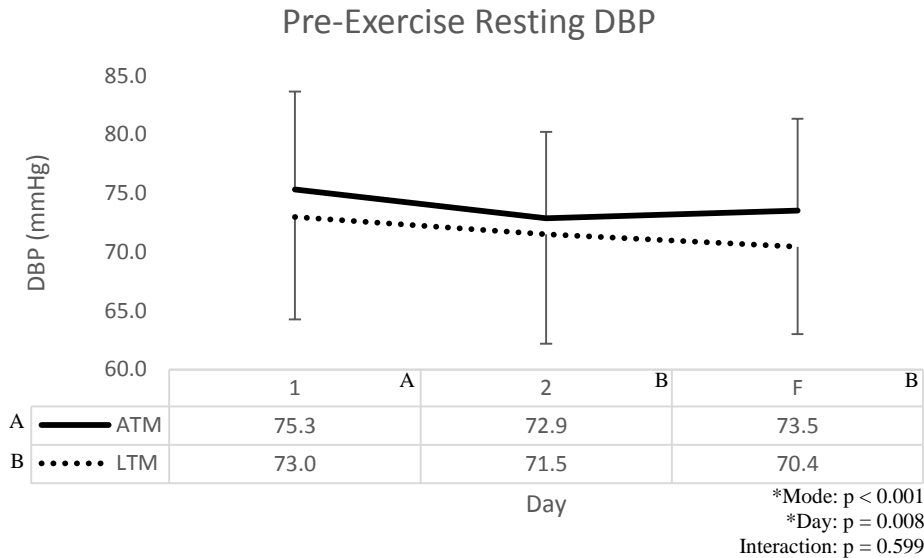


Figure 4.2. Resting diastolic blood pressure (DBP) prior to land (LTM) and aquatic (ATM) treadmill exercise. Measured on exercise days 1 and 2, and a third follow-up (F) day. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. $N = 19$. *main effect ($p < 0.05$). Differing letters (A, B) represent significant differences in post-hoc analysis for a given main effect. Dependent sample t-test revealed significant differences for ATM1 vs. LTM1, ATMF vs. LTMF, ATM1 vs. ATM2, and LTM1 vs. LTMF.

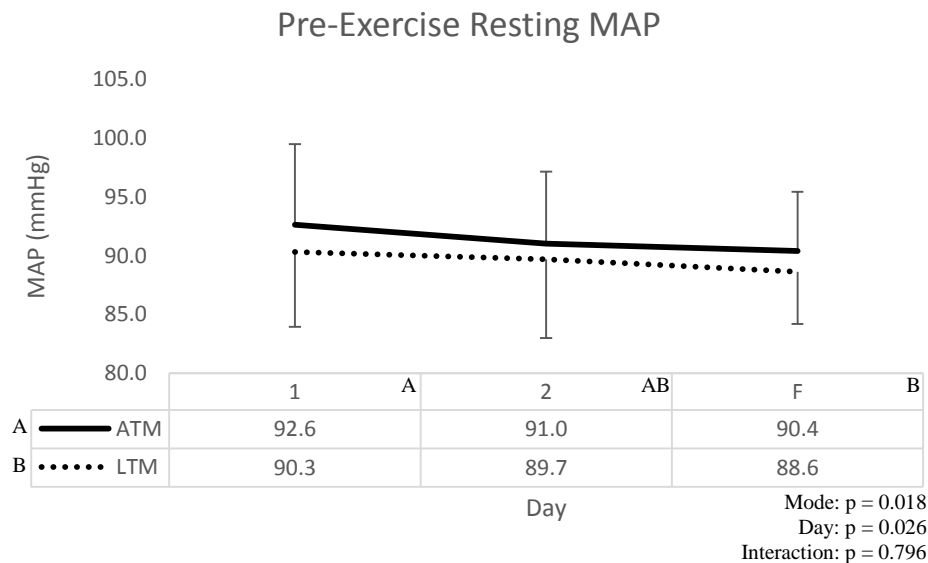


Figure 4.3. Resting mean arterial pressure (MAP) prior to land (LTM) and aquatic (ATM) treadmill exercise. Measured on exercise days 1 and 2, and a third follow-up (F) day. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. $N = 19$. Differing letters (A, B) represent significant differences in post-hoc analysis for a given main effect. Dependent sample t-test revealed significant differences for LTM1 vs. LTMF.

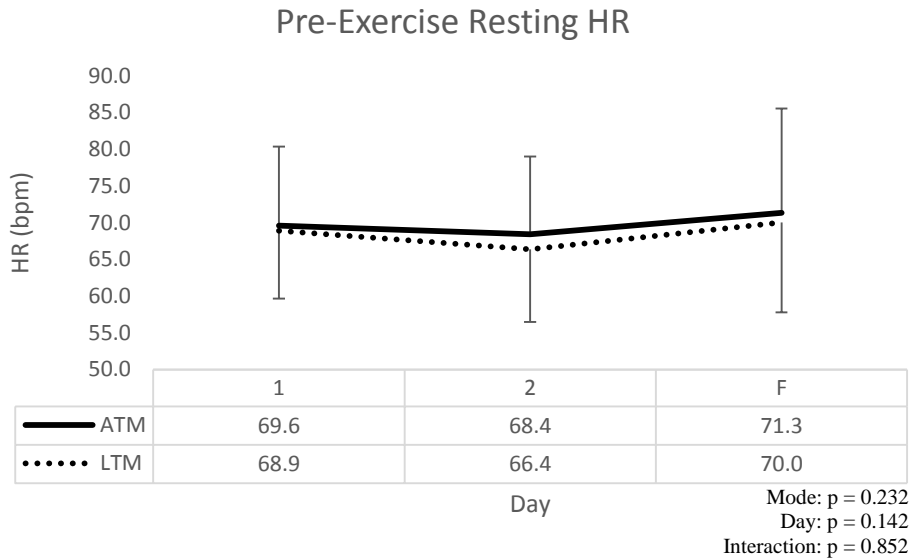


Figure 4.4. Resting heart rate (HR) prior to land (LTM) and aquatic (ATM) treadmill exercise. Measured on exercise days 1 and 2, and a third follow-up (F) day. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. $N = 19$.

Post-Exercise Blood Pressure

Following each exercise session, blood pressure was measured during seated recovery every 10 minutes from 20-60 minutes post-exercise. The average of these measures was calculated as the post-exercise blood pressure. To determine the magnitude of the post-exercise blood pressure reduction or hypotensive response, the pre-exercise blood pressure values were subtracted from the post-exercise values. The change in SBP, DBP, MAP, and HR following exercise are displayed in Figures 4.5-4.8, respectively. There were no significant main effects for mode or day for any of the measures. A paired sample t-test did reveal a trend ($p = 0.087$) for a lower 1-hour post exercise SBP following the day 1 aquatic treadmill exercise session (119 ± 10 mmHg) compared to resting, pre-exercise values (123 ± 9 mmHg) on the same day. This post-

exercise hypotensive response trend was not present for the other exercise days. The 95% confidence intervals for the change in SBP 1-hour post exercise were as follows: ATM1, 0.3 to -6.6 mmHg; ATM2, 1.4 to -4.7 mmHg; LTM1, 1.6 to -4.7 mmHg; LTM2, 0.9 to -3.5 mmHg.

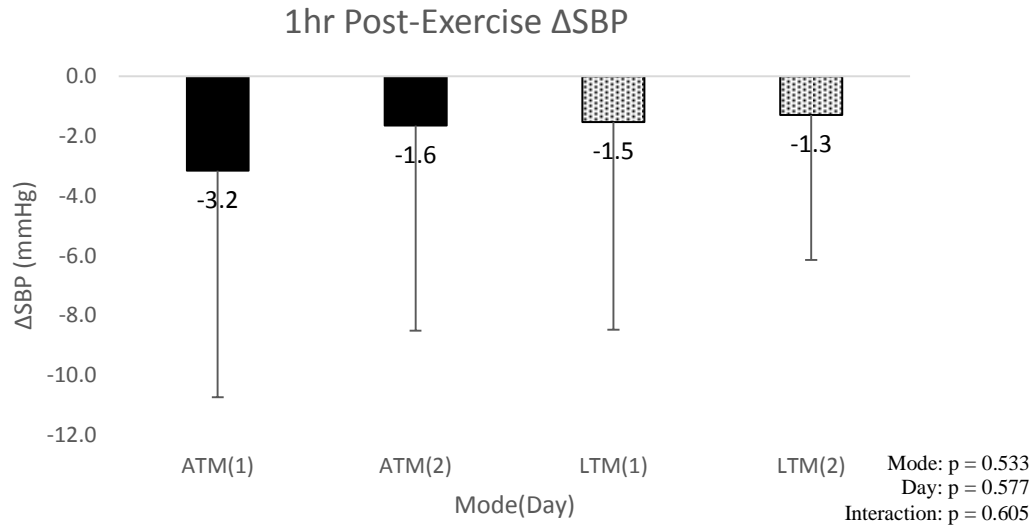


Figure 4.5. Difference in 1-hour post-exercise and pre-exercise systolic blood pressure (SBP). Post-exercise measures averaged from seated, resting measurements taken every 10 minutes from 20-60 minutes immediately post-exercise. Difference values calculated as post - pre. ATM = aquatic treadmill. LTM = land treadmill. (1) = Day 1. (2) = Day 2. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19.

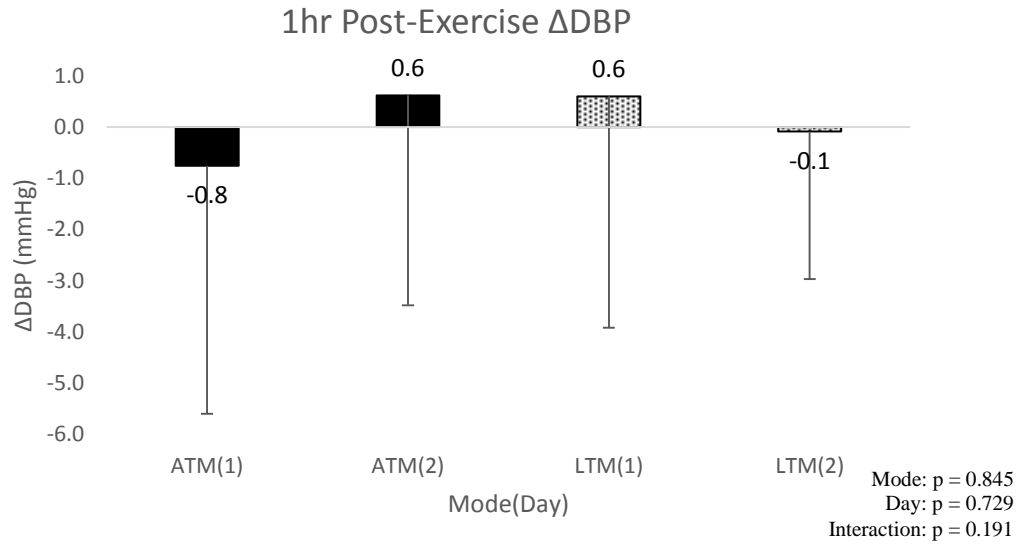


Figure 4.6. Difference in 1-hour post-exercise and pre-exercise diastolic blood pressure (DBP). Post-exercise measures averaged from seated, resting measurements taken every 10 minutes from 20-60 minutes immediately post-exercise. Difference values calculated as post - pre. ATM = aquatic treadmill. LTM = land treadmill. (1) = Day 1. (2) = Day 2. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19.

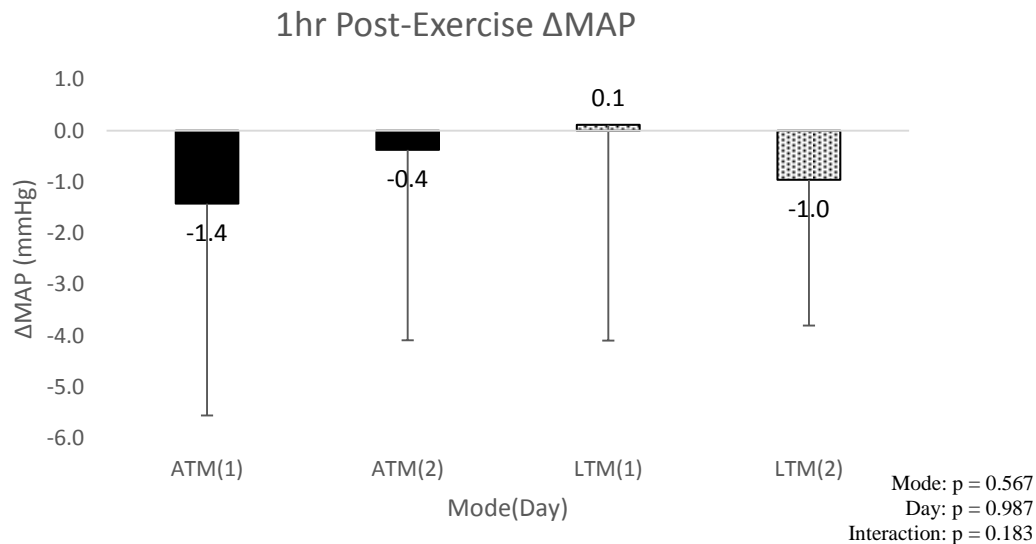


Figure 4.7. Difference in 1-hour post-exercise and pre-exercise mean arterial pressure (MAP). Post-exercise measures averaged from seated, resting measurements taken every 10 minutes from 20-60 minutes immediately post-exercise. Difference values calculated as post - pre. ATM = aquatic treadmill. LTM = land treadmill. (1) = Day 1. (2) = Day 2. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19.

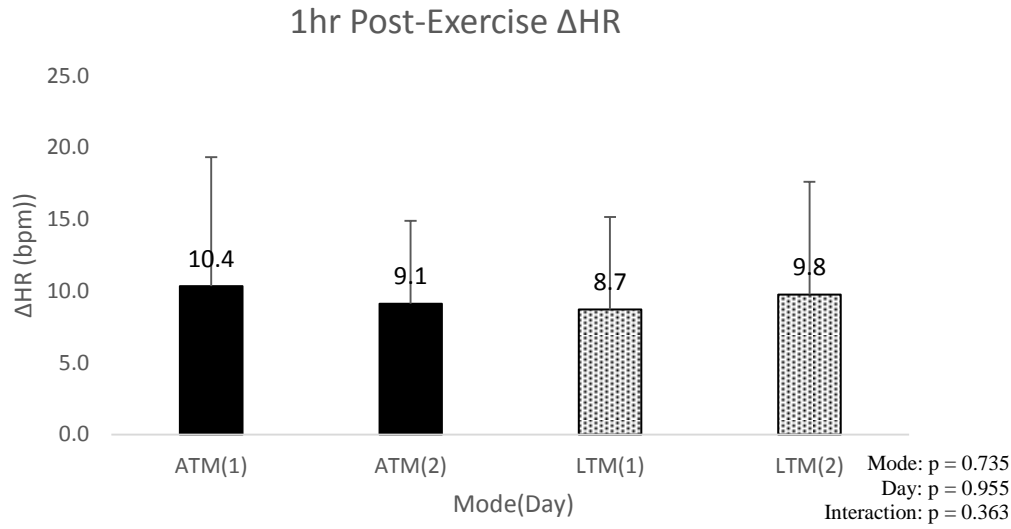


Figure 4.8. Difference in 1-hour post-exercise and pre-exercise heart rate (HR). Post-exercise measures averaged from seated, resting measurements taken every 10 minutes from 20-60 minutes immediately post-exercise. Difference values calculated as post - pre. ATM = aquatic treadmill. LTM = land treadmill. (1) = Day 1. (2) = Day 2. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19

Ambulatory Blood Pressure

Following each exercise session ambulatory blood pressure was measured every 20 minutes for the remainder of the day during waking hours. One subject did not participate in this portion of the study as there was not an adequately sized cuff available. Measures were averaged and are displayed for SBP, DBP, MAP, PP, and HR in Figures 4.9-4.13, respectively. While there were no significant main effects for day or mode on any of the measures, there was a trend ($p = 0.063$) for a lower DBP on day 2 (77 ± 7 mmHg) than day 1 (79 ± 9 mmHg).

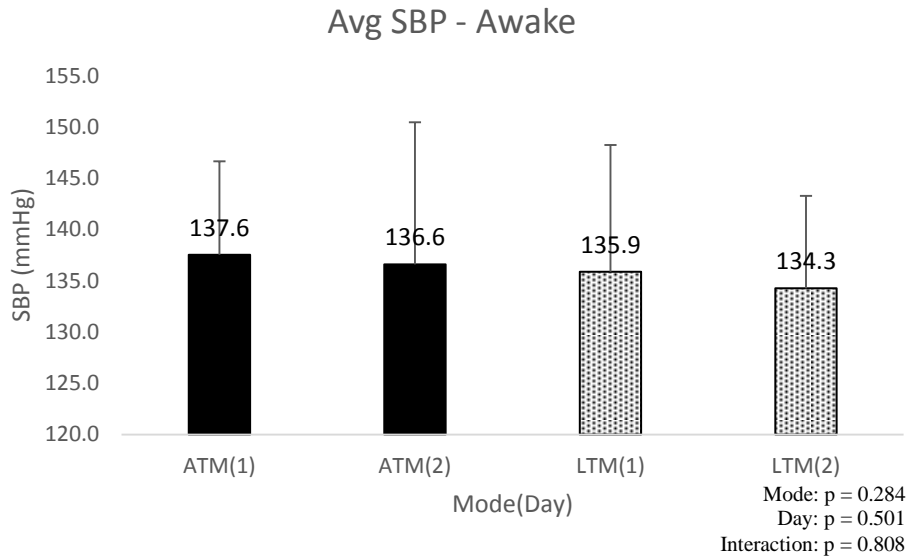


Figure 4.9. Average ambulatory systolic blood pressure (SBP) over waking hours following day 1 and 2 of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 18.

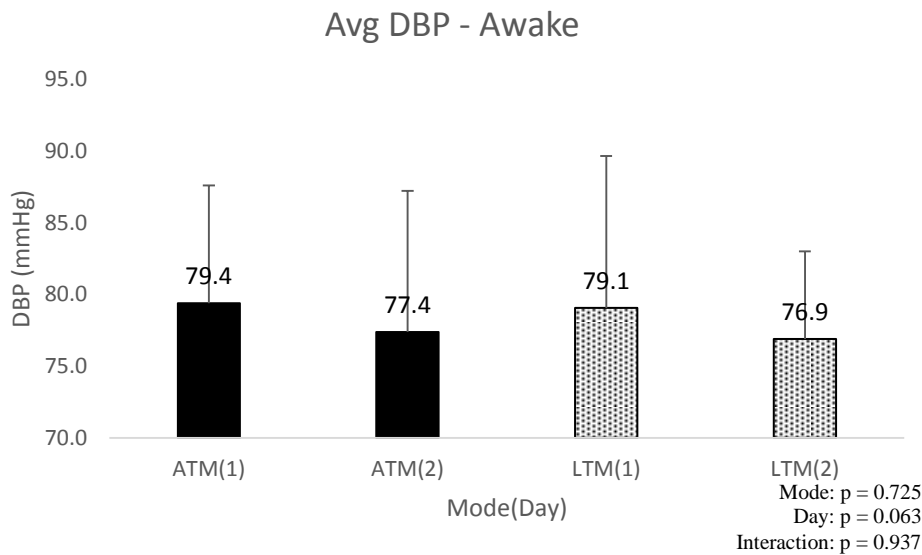


Figure 4.10. Average ambulatory diastolic blood pressure (DBP) over waking hours following day 1 and 2 of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 18.

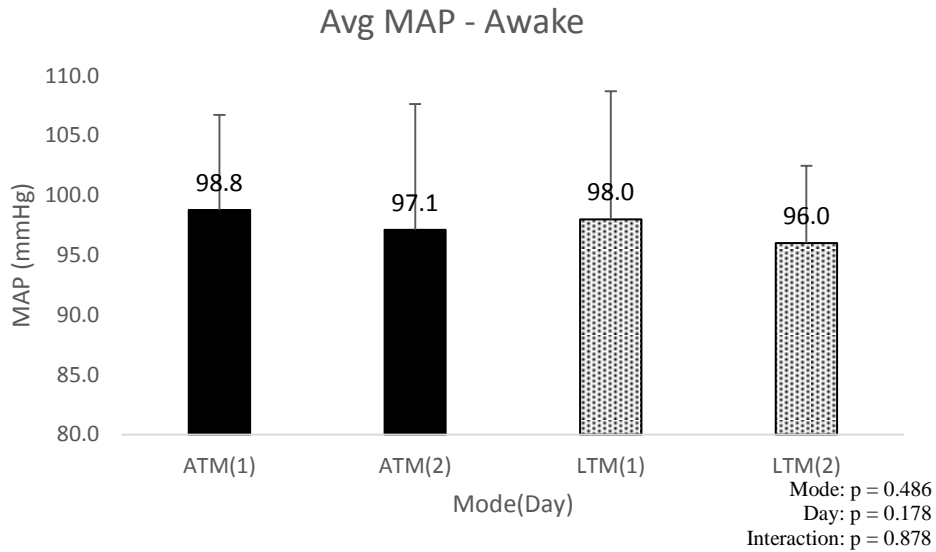


Figure 4.11. Average ambulatory mean arterial pressure (MAP) over waking hours following day 1 and 2 of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 18.

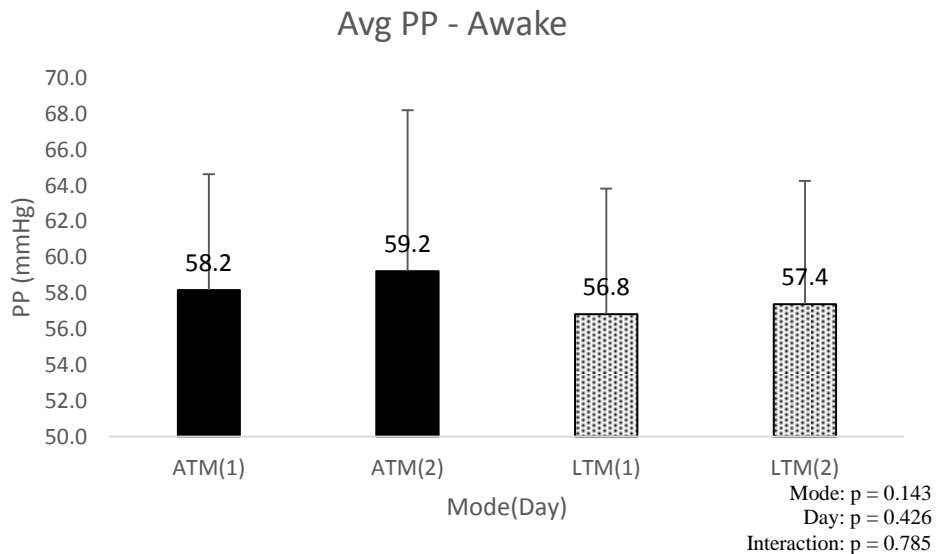


Figure 4.12. Average ambulatory pulse pressure (PP) over waking hours following day 1 and 2 of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 18.

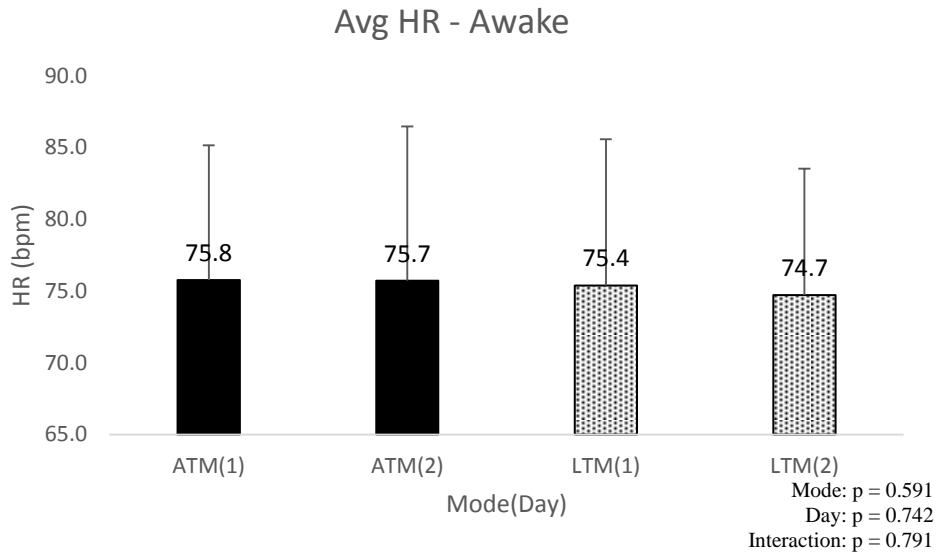


Figure 4.13. Average ambulatory heart rate (HR) over waking hours following day 1 and 2 of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 18.

Flow-Mediated Dilation

FMD was measured pre-exercise and 1-hour post exercise for the two consecutive exercise days and a third follow-up day for each mode. Results for FMD data are displayed in Figure 4.14. Analysis by a 2 (mode: ATM, LTM) \times 5 (time point: Day 1 pre-exercise, Day 1 1-hour post-exercise, Day 2 pre-exercise, Day 2 1-hour post-exercise, Day 3 follow-up) repeated measures ANOVA showed a trend ($p = 0.076$) for a main effect for mode. A dependent sample t-test revealed no difference in initial FMD measures (ATM1 Pre: $6.5 \pm 3.9\%$ vs. LTM1 Pre: $6.7 \pm 3.5\%$, $p = 0.872$) between modes, but a trend for a significant difference between modes 1-hour Post Exercise (ATM1 Post: $7.4 \pm 4.7\%$, LTM1 Post: $5.5 \pm 3.6\%$, $p = 0.051$). The change in FMD at 1, 24, and 48 hours following the initial exercise session for each mode is also displayed in Figure

4.15. There was no difference by mode or time point in the FMD flow stimulus, indicated by the change in post-occlusion brachial blood flow (Figure 4.16).

Pulse Wave Velocity

Carotid-femoral pulse wave velocity was also measured pre-exercise and 1-hour post exercise for the two consecutive exercise days and a third follow-up day for each mode (Figure 4.17). Analysis by a 2 (mode) x 5 (time point) repeated measures ANOVA showed a trend ($p = 0.056$) for a main effect in exercise mode. Dependent sample t-tests showed significantly greater ($p = 0.046$) PWV for ATM (6.7 ± 1.3 m/s) than LTM (6.2 ± 0.9 m/s) at time point 1, post-exercise. Furthermore, there was a trend for higher PWV ($p = 0.062$) at time point 2, pre-exercise for ATM (6.8 ± 1.4 m/s) than LTM (6.2 ± 0.8). However, there was no effect of mode or time point in the change in PWV calculated from time point 1, pre-exercise to 24 and 48 hours post-exercise (Figure 4.18).

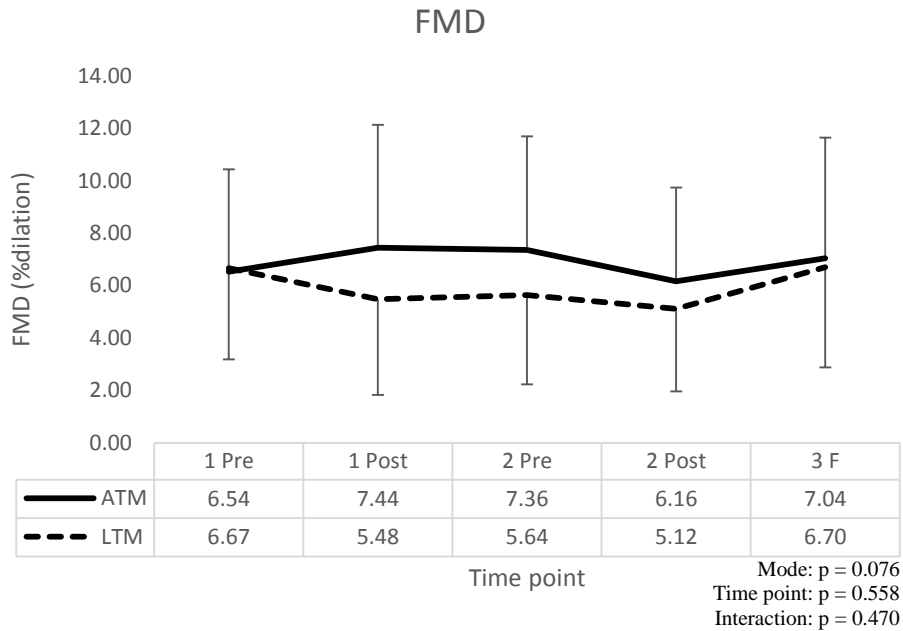


Figure 4.14. Flow-mediated dilation (FMD) pre and 1-hour post-exercise on two consecutive days of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days with a third follow-up day (F). Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19.

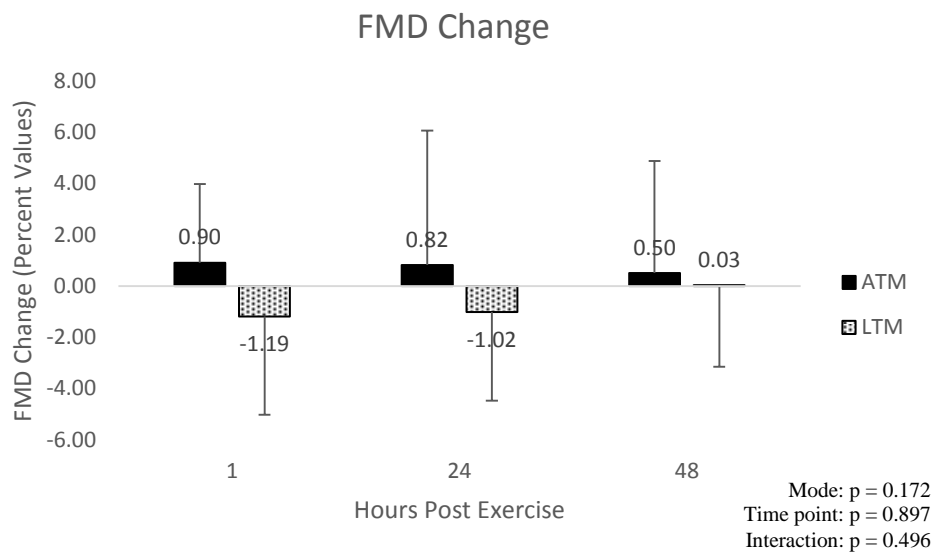


Figure 4.15. Change in flow-mediated dilation at 1, 24, and 48 hours following initial exercise session on two consecutive days of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days with a third follow-up day at 48 hours following initial session. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 19.

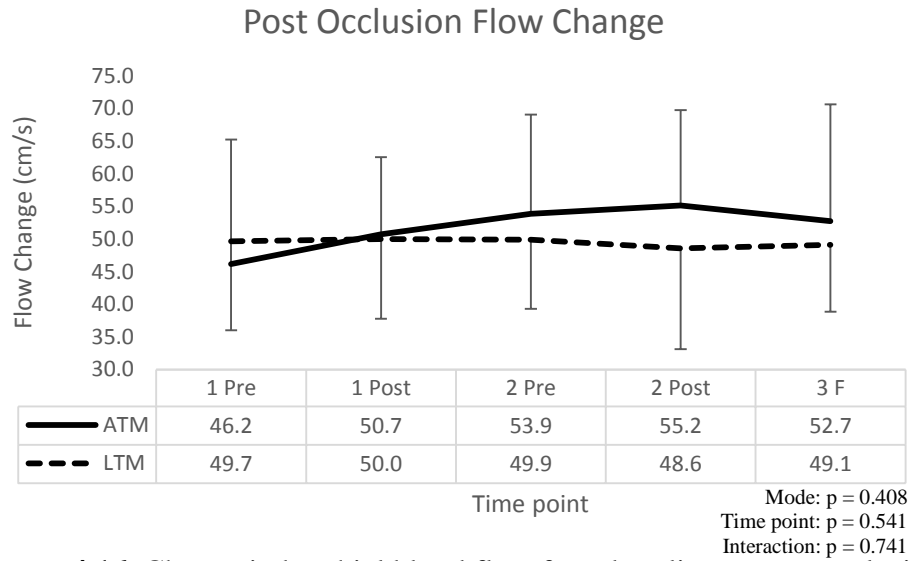


Figure 4.16. Change in brachial blood flow from baseline to post-occlusion during flow-mediated dilation. Procedure performed pre and 1 hour post-exercise on two consecutive days of aquatic (ATM) and land (LTM) exercise and a third follow-up day (F). Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. N = 15.

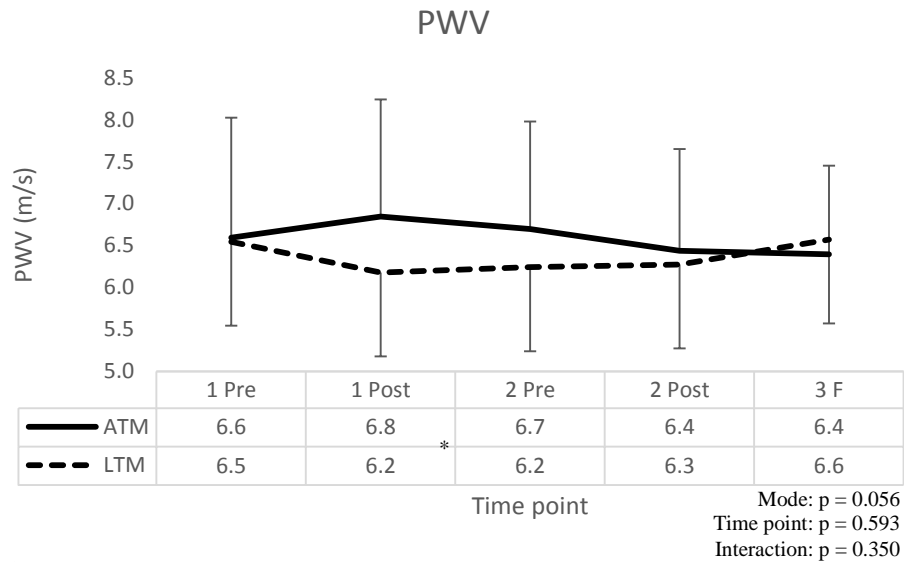


Figure 4.17. Carotid-femoral pulse-wave velocity (PWV) pre and 1-hour post-exercise on two consecutive days of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days with a third follow-up day (F). Cross-over design with 1-2 weeks between opposing exercise modes. * $p < 0.05$ between modes at a given time point in dependent sample t-test. Values represent mean \pm SD. N = 16.

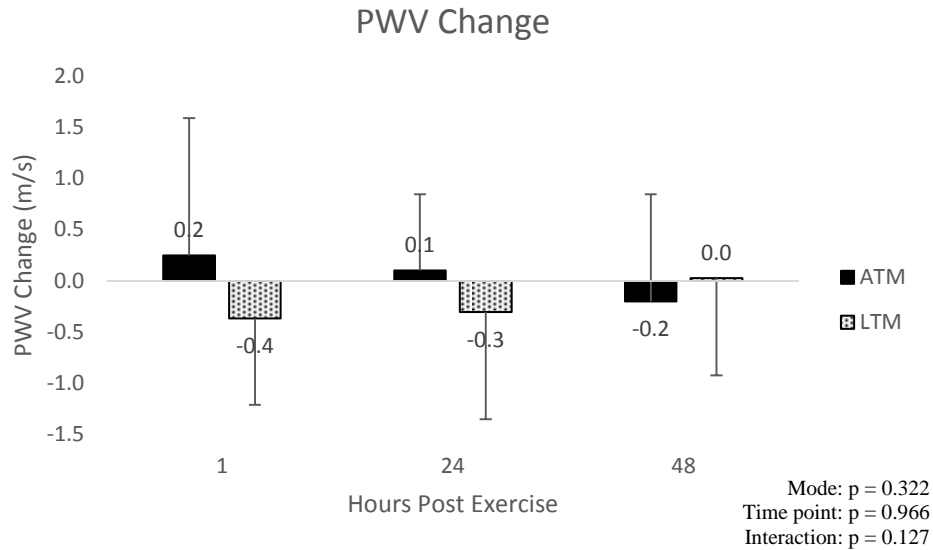


Figure 4.18. Change in carotid-femoral pulse-wave velocity (PWV) at 1, 24, and 48 hours following initial exercise session on two consecutive days of aquatic (ATM) and land (LTM) treadmill exercise. Exercise sessions for a given mode occurred on consecutive days with a third follow-up day at 48 hours following initial session. Cross-over design with 1-2 weeks between opposing exercise modes. Values represent mean \pm SD. $N = 19$.

Blood Analysis

Blood samples were taken on day 1 and 2 pre-exercise, immediately post-exercise, and 1-hour post exercise and on a third follow up day. Plasma nitrite/nitrate concentrations adjusted for changes in plasma volume showed no main effect for mode or time point (Figure 4.19). However there was a significant effect of time point on the percent change in plasma volume (Figure 4.20). Post-hoc analysis showed significant differences in the percent change in plasma volume for the following comparisons among time points: 1 IPE ($-3.0 \pm 3.4\%$) < 2 Pre ($3.1 \pm 5.4\%$), 2 IPE ($-0.4 \pm 4.7\%$), 3 F ($1.4 \pm 5.8\%$); 1 1hr ($-1.9 \pm 3.7\%$), 2 IPE ($-0.4 \pm 4.7\%$), 2 1hr ($-1.0 \pm 4.6\%$) < 2 Pre ($3.1 \pm 5.4\%$).

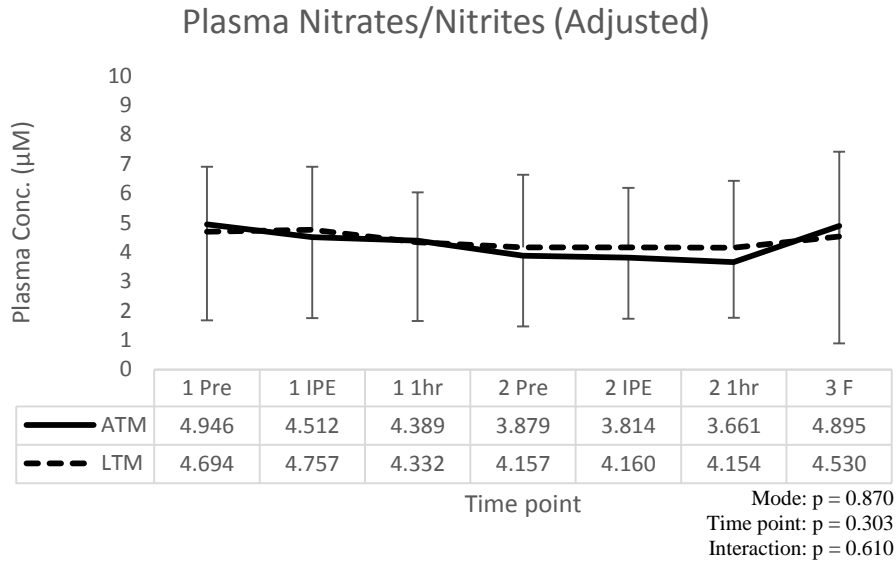


Figure 4.19. Plasma nitrates/nitrites adjusted for plasma volume. Measures taken at following time points: pre-exercise, immediately post-exercise (IPE), and 1 hour post-exercise on two consecutive days of aquatic (ATM) and land (LTM) exercise and a third follow-up day (F). Values represent mean \pm SD. $N = 13$.

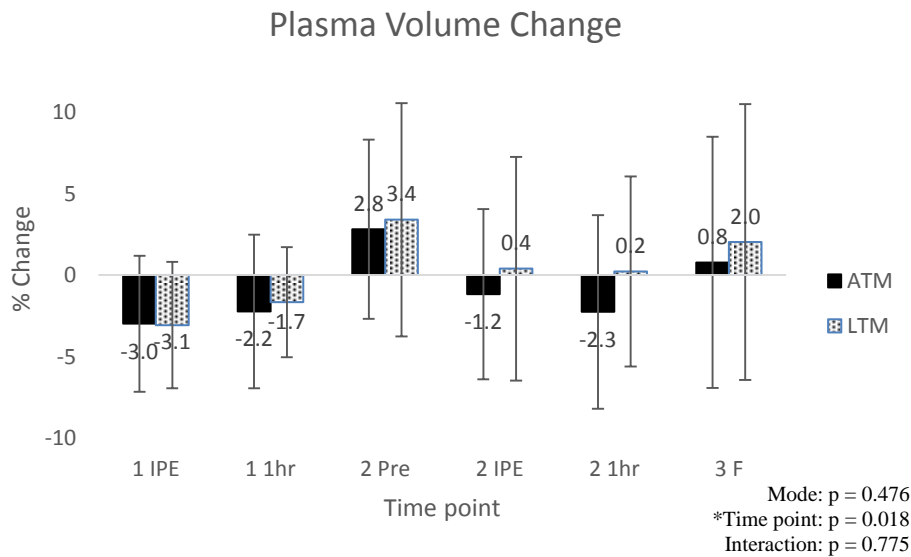


Figure 4.20. Percent change in plasma volume. Measures calculated relative to day 1 pre-exercise at the following time points: immediately post-exercise (IPE) and 1 hour post-exercise on two consecutive days of aquatic (ATM) and land (LTM) exercise and a third follow-up day (F). Values represent mean \pm SD. $N = 13$.

Diet Analysis

Dietary records were completed in full for each of the three-day sequences by 13 of the 19 subjects. Daily averages for macronutrients and micronutrients of interest or significant difference are displayed in Table 4.1. While subjects were instructed to eat similar diets during these two periods, protein intake was lower ($p = 0.035$) for ATM (72 ± 22 g) than LTM (96 ± 34 g). Because of this, most of the amino acids were also lower for ATM than LTM, including Arginine (ATM: 2 ± 1 mg, LTM: 3 ± 2 , $p = 0.033$). There were no differences in sodium or omega-3 fatty acid intake. Dietary logs did confirm that subjects abstained from alcohol and caffeine.

| Variable | ATM | | LTM | | P-Value |
|------------------|------|------|------|------|---------|
| | Mean | SD | Mean | SD | |
| Energy (Kcal) | 1533 | 501 | 1748 | 586 | 0.132 |
| Protein (g) | 72 | 22 | 96 | 34 | *0.035 |
| Carbs (g) | 185 | 72 | 195 | 69 | 0.672 |
| Sugars (g) | 59 | 32 | 58 | 33 | 0.899 |
| Fiber (g) | 15 | 5 | 15 | 7 | 0.902 |
| Fat (g) | 58 | 23 | 66 | 30 | 0.239 |
| Sat Fat (g) | 19 | 8 | 23 | 14 | 0.165 |
| Trans Fat (g) | 1 | 2 | 1 | 3 | 0.208 |
| Mono Fat (g) | 14 | 6 | 18 | 10 | 0.208 |
| Poly Fat (g) | 8 | 4 | 6 | 3 | 0.223 |
| Omega 3 (g) | 1 | 1 | 1 | 1 | 0.672 |
| Omega 6 (g) | 5 | 2 | 4 | 2 | 0.374 |
| Cholesterol (mg) | 266 | 186 | 359 | 263 | 0.259 |
| Sodium (mg) | 2568 | 1070 | 2768 | 1411 | 0.431 |
| Potassium (mg) | 1634 | 676 | 1972 | 1060 | 0.372 |
| Iron (mg) | 9 | 5 | 13 | 6 | *0.014 |
| Arginine (mg) | 2 | 1 | 3 | 2 | *0.033 |

Table 4.1. Diet analysis

CHAPTER V

DISCUSSION

Hypothesis and Specific Aims

The purpose of this study was to test the hypothesis that compared to traditional land treadmill exercise, an acute bout of aquatic treadmill exercise elicits a more favorable post-exercise vascular response in flow mediated dilation, pulse wave velocity, plasma nitrite levels, and resting and ambulatory blood pressure in sedentary, pre-hypertensive men.

Aim 1: In terms of pre-exercise resting blood pressure, the post-exercise hypotensive response, and ambulatory blood pressure, we reject the hypothesis that ATM exercise would lower blood pressure to a greater degree than LTM exercise.

Aim 2: In line with our hypothesis, there was a trend ($p = 0.076$) for a more favorable augmentation in the FMD response with ATM than LTM exercise (Figure 4.14).

Aim 3: We reject the hypothesis that ATM exercise would elicit a greater increase in plasma nitrates/nitrites than LTM exercise.

Aim 4: Contrary to our hypothesis, there was a trend ($p = 0.056$) for a more favorable attenuation in PWV with LTM than ATM exercise (Figure 4.19).

Resting Blood Pressure

While there was a main effect for mode with greater resting DBP and MAP for ATM, the difference between ATM and LTM were present from the day 1 measure prior to any exercise intervention. The differences persisted at day 2 and the 3rd follow up day making for a mode specific difference in the analysis. However, the relative decreases in

blood pressure across days were similar between modes, indicating that the influence of the exercise intervention was not different between modes. More importantly, there was a main effect for Day on both DBP and MAP. DBP was lower on day 2 and 3 than day 1, and MAP was lower on day 3 than day 1. Chronic exercise training has been shown to reduce resting SBP by 7 mmHg on average (85). A recent meta analysis indicated that reductions in blood pressure were greatest in hypertensive men exercising at moderate to high intensities for 30-45 minutes and less than 210 minutes per week for less than 12 weeks (16). While these reviews (16, 85) would indicate that a longer training intervention is necessary to reduce resting blood pressure, the current results show that there was a significant effect of the acute exercise sessions on reducing resting blood pressure 24 and 48 hours later. This short term reduction in resting blood pressure with acute exercise is however consistent with previous findings in healthy subjects following 1-week and 3 sessions of cycle exercise for 40 minutes at 60-70% maximal work capacity where subjects on average had a reduction in SBP and DBP of 8 mmHg and 5 mmHg, respectively (69). While there was no control condition in the present experiment to compare the exercise intervention, as the main interest was to make mode to mode comparisons, it is unlikely that the reduced blood pressure measurements are due to familiarization alone. Subjects had reported to the lab on two separate occasions prior to the acute exercise sessions where they underwent the resting blood pressure screening procedures. Furthermore, there was a general decline seen across all three days of acute sessions, not just from day 1 to 2. These findings are significant as while there were not any mode specific differences in the effect on resting blood pressure, acute

exercise sessions on two consecutive days were effective at lowering resting blood pressure by ~2-2.5 mmHg in the 24-48 hours following the initial exercise session in physically inactive, pre-hypertensive men.

Elevated blood pressure, while a disease in itself, is also a significant risk factor for cardiovascular disease (27). According to the Centers for Disease Control and Prevention (CDC), more than 600,000 americans, die of heart disease every year (55). Even though the men in the present study were not hypertensive and their systolic blood pressure was generally in the 125-130 mmHg range, a reduction in resting BP for these individuals is still beneficial to cardiovascular disease risk. A recent press release by the National Institute's of Health (NIH) announced that a landmark trial revealed that treating hypertensive patients to get to a target SBP of 120 mmHg rather than 140 mmHg resulted in a 1/3 reduction in cardiovascular events (76). Therefore there appears to be benefit in reducing blood pressure even below pre-hypertensive ranges in terms of cardiovascular risk. The rate of disease reduction increases in proportion to blood pressure reduction, but reductions in DBP as little as 4 mmHg have been shown to reduce CVD risk by up to 50% (39). In addition to the benefits of reduced DBP, reductions in SBP are also reflected in the rate-pressure product and reducing the work load placed on the heart. Many of the acute benefits of exercise accumulate into the observed chronic physical fitness adaptations (64). Overall, a reduction in resting blood pressure, as observed in the current study, and carried out over time could have significant health ramifications in managing risk of hypertension and cardiovascular disease.

Post-Exercise Hypotension

While there were no main effects for mode or day on the magnitude of the post-exercise hypotensive response, a dependent sample t-test did reveal a trend ($p = 0.087$) towards significant differences in the pre-exercise SBP (123 ± 9 mmHg) and the 1-hour post-exercise SBP (119 ± 10 mmHg) for the ATM1 session only. In a previous pilot study we observed a trend ($p = 0.059$) towards a lower post-exercise SBP in the 2-hours following ATM (113 ± 10 mmHg) than LTM (116 ± 8 mmHg) exercise in recreationally active college men exercising at $\sim 75\%$ of maximal heart rate. Similarly, both trained and untrained, normotensive women showed greater reductions in blood pressure 1-hour following water-walking than land-walking at only 40% $\text{VO}_{2\text{max}}$ (92). While there was a trend for significant differences between pre and post-exercise SBP following the initial aquatic treadmill session in the present study, there were no mode specific differences between ATM and LTM as analyzed by the whole statistical model. The fact that exercise intensity in the present study (60% $\text{VO}_{2\text{max}}$) was slightly lower compared to our previous pilot study in recreationally active college men (75% max heart rate) may play a role as the magnitude and duration of the post-exercise hypotensive response has been shown to increase with higher exercise intensities (50% vs 75% $\text{VO}_{2\text{max}}$) (89). The time of day may also have played a role in the magnitude of the post-exercise hypotensive response. Each subject reported to the lab at the same time of day for each session in the 3-day sequences, which occurred anywhere from 5:00-10:00am. It was previously shown that the magnitude of the hypotensive response following exercise was attenuated in the morning, likely due to a less apparent decrease in peripheral resistance

following exercise in the morning (48). It was clarified by de Brito et al. (19) that when the post-exercise hypotensive response following morning exercise was adjusted for a control session and the normal circadian rise in blood pressure over that time period, morning exercise actually resulted in a greater post-exercise hypotensive response than afternoon exercise. The reason for the post-exercise blood pressure reduction was accredited primarily to a reduced cardiac output, secondary to a reduction in peripheral vascular resistance (19). While there were no control sessions to adjust the post-exercise hypotensive response in the present study, it is likely that the small magnitude of BP reduction observed in our study was a result of the morning exercise period. It is possible that if there were small changes between modes, these could be magnified by adjusting for a control session in the morning or observing the mode specific response in an afternoon exercise session. Training status, gender, hypertensive status, and the method of determining the post-exercise hypotensive response are also confounding factors in making comparisons between studies. In our present study we cannot conclude any mode or day dependent differences, but there was a trend towards a greater SBP reduction following ATM1 (-3.2 ± 7.6 mmHg) that was not seen in the other sessions (ATM2: -1.6 ± 6.8 mmHg, LTM1: -1.5 ± 6.9 mmHg, LTM2: -1.3 ± 4.8 mmHg).

Ambulatory Blood Pressure

There were no main effects for mode or day for any of the ambulatory measurements, although average ambulatory blood pressures did tend to decrease on exercise day 2 compared to day 1. While we do not have a control condition to definitively conclude that a second day of exercise resulted in a reduction in ambulatory

blood pressure, this trend existed despite the counterbalanced design of the present study. For instance, while it could be argued that there is a learning effect of wearing the ambulatory cuff that resulted in lower averages on day 2 than day 1 initially, the fact that all subjects repeated this for each mode means that this trend persisted even with the second mode. Essentially the lower day 2 blood pressures persisted even from the 3rd to the 4th time that subjects wore the device. Therefore, there may be a cumulative effect of exercise on ambulatory blood pressure in previously inactive, pre-hypertensive men. As there is no literature on repeated use of ambulatory blood pressure on multiple days, these data are novel nonetheless. It should also be noted that following exercise, these inactive, pre-hypertensive men did on average have ambulatory blood pressures near normal recommended ranges (135/85 mmHg) (78). This is significant as ambulatory blood pressure is a valuable predictor of CVD beyond traditional markers (78, 118). A meta-analysis has previously shown reductions in SBP and DBP of approximately 3 mmHg on average (15). The duration of the reduction in ambulatory pressure may also be intensity dependent, as intensities of 75% VO_2max resulted in a longer lasting impact than 50% VO_2max (89). Given that our study matched intensities between aquatic and land sessions, this is not as much of a concern, but the relatively moderate intensities (60% VO_2max) could have resulted in less of an effect overall if there were any differences. Our findings of similar ambulatory blood pressures following both aquatic and land treadmill exercise are comparable to previous results showing equivalent post-exercise ambulatory blood pressures following both aquatic and land aerobic exercise in pre-hypertensive and hypertensive men and women (108). However, in the previously

mentioned study, participants completed approximately 1-hour of water aerobic exercise for the aquatic session and completed a similar bout of aerobic and circuit type training for the land session. While their average heart rates were similar for both modes, actual energy expenditure by indirect calorimetry was not confirmed between sessions as in our present study. However, Terblanche et al. (108) did compare the aquatic and land aerobic exercise to a control session in the same subjects and showed SBP to be reduced by approximately 10 mmHg on average with the exercise sessions (108). While we did not have a control session in the present study, our findings demonstrate that, in pre-hypertensive men, ambulatory blood pressures are comparable and within normal ranges following both aquatic and land treadmill exercise. These results justify the promotion of aquatic exercise in the maintenance of normal day-time ambulatory blood pressure, which is an important cardiovascular risk factor.

Flow-Mediated Dilation

There was a trend for an overall main effect for mode ($p = 0.076$) on FMD, and despite no difference in FMD between ATM1 and LTM1 prior to exercise, ATM1 tended to have a greater FMD than LTM1 1-hour post exercise. These findings are partially in line with our hypothesis that FMD would be augmented post-exercise to a greater degree with ATM than LTM. There were however no differences in FMD between modes at the day 3 follow-up point. An augmentation in FMD is indicative of enhanced endothelial function, which is beneficial to cardiovascular health (99). Furthermore, FMD is a valuable health marker as it has been shown to correlate poorly with traditional CVD markers, making it a useful independent indicator of future

disease development (65). While short term aerobic exercise training over the course of approximately 12 weeks has been shown to enhance FMD (20, 33, 99), there is less conclusive evidence concerning the acute effects of exercise on the FMD response. Much of this inconsistency is due to variation in the population studied, the mode, duration, and intensity of exercise, and the time that FMD is measured post exercise. In healthy males, cycling at higher intensities (70 and 85% max HR) for 30 minutes resulted in impaired FMD immediately post exercise, whereas there was no change in FMD at low intensities (50% max HR) even when baseline diameter and shear were controlled for post-exercise (7). In the present study, intensity was moderate and matched between modes. In overweight men who were active, an acute bout of exercise, regardless of exercise intensity, augmented FMD one hour post exercise, whereas FMD was attenuated in the inactive subjects (40). In our study, subjects were all physically inactive and all subjects completed both exercise modes. The total exercise dosage or caloric expenditure is also a concern, as it has been shown in healthy, active men that acute exercise, regardless of intensity, in a total dosage equivalent to 30 minutes at 50% max resulted in improvements in FMD immediately post exercise and at one and two hours post. However, FMD either remained the same or was attenuated at both high and low intensities when total work and duration were increased (47). Our study required subjects to exercise at 60% VO₂max for a duration of 300 kcal, which is similar to the duration and intensity in the Johnson et al. (47) study which showed augmented FMD at 50% VO₂max for 30 minutes. There are a number of potential mechanisms that could contribute to enhanced FMD, including: increased NO availability, decreased oxidative

stress, or an increase or decrease in other vasodilatory (prostacyclin, endothelial derived relaxing factors) and vasoconstrictor molecules (endothelin-1), respectively (99). While we did not find any differences between ATM and LTM in plasma nitrites/nitrates, as a marker of NO availability, these other vasoactive molecules may be the cause of the observed FMD changes and should be investigated in future research.

Plasma Nitrites/Nitrates

As mentioned, the observed increased in FMD following ATM exercise was in line with our hypothesis. Previous research has shown that chronic ATM training results in greater eNOS content in skeletal muscle biopsies (57). Given that eNOS is responsible for NO synthesis in the endothelium, we measured total plasma nitrites/nitrates as a marker of NO availability following the acute exercise in the present study. There were however no differences observed between modes for this blood marker. It has previously been demonstrated that increases in plasma nitrite more closely mimic the activity of eNOS than does plasma nitrate (59). Because the blood analysis we performed converted all nitrate to nitrite to measure total nitrate/nitrite, we are unable to distinguish the proportion of each. Other studies have shown that chronic exercise training was effective at increasing basal NO production, but these studies measured the difference between arterial and venous blood (53, 63). In an acute exercise model, total plasma nitrates/nitrates were increased following two days of exercise in cardiac patients. While these investigators utilized venous measurement and NO analysis similar to the present study, it may be that the differences in subject populations played a role in the response

as it was hypothesized that ischemic preconditioning played a role in an augmented NO response following a second acute exercise session (126).

In addition to the oxidized forms (Nitrate/Nitrite) of NO found in the lumen, endothelial NO can also undergo nitrosylation or nitrosation (54). Given we only measured total plasma nitrate/nitrite from venous blood draws in the present study, we are unable to conclude differences in net NO production and consumption or determine the quantity of other endothelial NO metabolites that reached the lumen. Furthermore, in addition to NO there are other previously mentioned vasoactive compounds, such as prostacyclin, that could be responsible for the presently observed changes in FMD. It should be noted that only 13 of the 19 subjects were included in the blood analysis as a sample was not able to be obtained at all 14 time points on all of the subjects.

Pulse Wave Velocity

Contrary to our hypothesis, there tended to be a more favorable response, or attenuation, in PWV following LTM exercise than ATM exercise. PWV decreased from LTM1 pre-exercise (6.5 ± 1.1 m/s) to LTM1 1-hour post-exercise (6.2 ± 0.8 m/s) and LTM1 24-hour post-exercise (6.2 ± 0.9 m/s). On the other hand, PWV increased from ATM1 pre-exercise (6.6 ± 1.4 m/s) to ATM1 1-hour post-exercise (6.8 ± 1.4 m/s) and ATM1 24-hour post-exercise (6.7 ± 1.3 m/s). PWV was not different between ATM1 and LTM1 prior to exercise, but PWV was lower for LTM1 than ATM1 24-hours following exercise, as revealed by a paired sample t-test at that time point. It is unknown if the magnitude of these changes in PWV would be physiologically significant as a marker of arterial stiffness, as these values still fall within normal ranges of less than 7

m/s (1). Others have shown similar reductions in PWV of approximately 0.2 m/s up to 1-hour post-exercise following 1-hour of low intensity cycling (70). While acute exercise may cause a short-term temporary change in PWV, long-term changes in arterial stiffness and PWV are linked to more chronic training. Aerobic walking over the course of 3 months was effective and decreasing arterial stiffness in sedentary, middle-aged men (107). Both structural adaptations (elastin, collagen) in the vessel and functional changes due to vascular smooth muscle tone play a role in arterial compliance. Vascular smooth muscle tone can be increased by an increase in α -adrenergic stimulation, reduced NO availability, increased endothelin-1 and angiotensin II (96). Since we did not detect differences between modes in plasma nitrites/nitrates, perhaps sympathetic stimulation or some of these other vasoactive compounds played a role in the observed PWV response. However, as previously mentioned, it is unknown whether or not the small changes observed would be physiologically significant.

Dietary Analysis

Daily protein intake was higher for LTM (93 g) than ATM (72 g), which also resulted in larger amounts of arginine for LTM (3 mg) than ATM (2 mg). Arginine is utilized in the synthesis of NO, but the link between its supplementation and enhanced endothelial dependent dilation has not been consistently found (45). Furthermore, the quantities from the diet in the current analysis are much smaller than doses used with supplementation of multiple grams per day (45). Given these small values and the fact that FMD was actually augmented in ATM and attenuated in LTM, it is unlikely that these small differences in dietary arginine had any role in the results. It should also be

reiterated that all subjects reported to the lab at least 8 hours fasted for all sessions, and this was confirmed verbally and by dietary logs.

Limitations

Subject Compliance

As with many human studies, subject compliance could have impacted results if guidelines were not followed. Subjects were asked to abstain from physical activity, caffeine, alcohol, nicotine and other stimulants or supplements the day before study visits and for the 3-day study sequences. Subjects were reminded by email prior to all sessions on the next sessions requirements (fasted, no caffeine, etc.) and reminded prior to leaving each session. Compliance was confirmed verbally prior to each session. Ambulatory blood pressure data collection could have been affected if subjects did not follow procedures or wear cuff regularly and properly. Subjects were asked at each session if they had met compliance requirements.

Subject Scheduling

Subjects were required to report to the lab for 3-consecutive days on two separate occasions at approximately the same time for the exercise visits. While subjects were scheduled at the same time of day and on the same days, changes in their weekly schedule outside the confines of the study could have impacted results. Subjects were required to come to their acute exercise session visits in the morning, while fasted. Because of subject work and class schedules, some subjects had to arrive as early as 5:00 am for their 3 hour lab visits. The time of day may have resulted in lower pre-exercise blood pressures and blunted the magnitude of the observed post-exercise hypotensive

response relative to these low resting levels. Furthermore, the normal circadian rise in blood pressure over the morning period may have impacted the observed impact of the exercise session on blood pressure (48).

Delimitations

Subject Specificity

Recruitment criteria consisted of physically untrained, pre-hypertensive men between the ages of 18-65 who were not currently taking blood pressure medication. Screening for physical activity consisted of written and verbal questioning of current exercise habits. Screening for blood pressure required subjects to report to the lab on two separate occasions to determine if they meet the pre-hypertensive criteria (SBP: 121-139 mmHg and/or DBP: 81-89 mmHg). Subjects were verbally asked whether or not they had abstained from exercise, caffeine, alcohol, and nicotine on the day of screening and if they had avoided eating within 2 hours prior to screening.

Dietary Control

While subjects were asked to abstain from alcohol, nicotine, caffeine and other stimulants the day before and during the 3-day study sequences and they were required to come in fasted prior to each visit involving a blood draw and ultrasound measurement, their daily diet was not controlled otherwise. They were required to record all food and drink intake and encouraged to eat similarly on the following 3-day sequence.

Exercise Prescription

Subjects exercised at 60% of maximal aerobic capacity, as determined from the maximal treadmill graded exercise test, for a duration required to expend 300 kcal.

Varying intensities and durations of exercise could have different effect on post exercise FMD and blood pressure responses. Because these subjects were untrained, this intensity and duration was selected and the focus was placed on the mode comparison. The exercise order was randomized between subjects so that there were a balanced number of subjects beginning on the aquatic treadmill and on the land treadmill.

Blood Analysis

The analysis ran to determine total NO measured the sum of plasma nitrates/nitrites. Nitrites may be a better marker of eNOS activity, but we were not able to separate the two markers from the current analysis. Furthermore, there are alternate fates of NO in the lumen that were not measured in the current study.

Gender

Because the FMD response in women can be affected by time point in the menstrual cycle, the study population in the present investigation was delimited to men.

CHAPTER VI

CONCLUSIONS

General

This was the first study to compare the effects of both aquatic and land treadmill exercise on both blood pressure and arterial function. Our findings indicate that resting blood pressure, post-exercise blood pressure, and ambulatory blood pressure exhibit similar responses to acute bouts of aquatic and land treadmill exercise in physically inactive, pre-hypertensive men. Both modes were effective at decreasing resting DBP and MAP in the 24-48 hours following each initial exercise bout. Furthermore, in this group of men with pre-hypertensive resting blood pressures, ambulatory blood pressures fell near normal ranges (135/85 mmHg) following both ATM and LTM exercise.

In terms of arterial function, there was a trend for enhanced FMD at 1-hour and 24-hours post-exercise following ATM exercise, but not LTM exercise. Enhanced endothelial function as indicated by augmented FMD fell in line with our hypothesis based on previous findings indicating increased eNOS content in skeletal muscle following chronic ATM training (57). However our acute data measuring plasma nitrites/nitrates did not show any differences between modes, indicating that either NO availability may not have been the cause of the improvements in FMD or our method of assessing NO was inadequate. Nonetheless, an augmentation in FMD is considered a favorable response, as FMD is a marker of endothelial function and is related to both blood pressure regulation and CVD risk. Contrary to our findings with FMD as a marker

of improved arterial health, ATM exercise tended to demonstrate a less favorable response in PWV or arterial stiffness than LTM exercise. However, whether these changes have physiological relevance is unknown, as the changes were small (~ 0.2 m/s) and PWV was still within normal ranges (< 7 m/s).

Future Research

In the current investigation, exercise intensity was set relatively low (60% $\text{VO}_{2\text{max}}$) as the subject population was untrained. Since the magnitude and duration of the post exercise blood pressure response and ambulatory blood pressure can be impacted by exercise intensity, future research might explore higher intensities than those utilized in the current study to magnify any potential differences between modes. A greater magnitude in the blood pressure responses may also be observed in the afternoon or by adjusting for a morning control session. Furthermore, identifying potential mechanisms behind the observed changes in FMD and PWV could be a target of future research. Since NO availability as indicated by plasma nitrates/nitrites was not different between modes, other plasma NO metabolites and endothelial vasodilators, such as prostacyclin and vascular endothelial growth factors could be potential candidates for the differential FMD response observed in the current study. Finally, the small changes in PWV observed could potentially be explored through differences in autonomic regulation following exercise.

Significance

Overall, these findings suggest that acute bouts of aquatic treadmill exercise are at least equally beneficial as land treadmill exercise on impacting blood pressure in

sedentary, pre-hypertensive men. In some cases, such as the observed augmentation in flow-mediated dilation, aquatic treadmill exercise may offer an additional benefit beyond that of land treadmill exercise. In conjunction with previous findings of improved cardiovascular health, blood pressure reactivity, lean mass, muscle strength, and muscle protein synthesis with aquatic treadmill exercise, these findings support the use of aquatic treadmill exercise for the promotion of health-related fitness and prevention of CVD risk (35, 57, 58).

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APPENDIX A

CONSENT FORM

Vascular Responses to Acute Land and Aquatic Treadmill Exercise in Pre-Hypertensive Men

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. If you decide to participate in this study, this form will also be used to record your consent.

The purpose of the present study is to determine the acute vascular responses to aquatic and land treadmill exercise.

What will I be asked to do?

Your participation is completely voluntary.

Following consent, you will come to the Applied Exercise Science Lab for 7 visits. A sample study timeline is provided in Figure 1. The first visit will consist of a maximal graded exercise test (GXT) to assess cardiovascular fitness and a DEXA body composition scan. Approximately 1 week later, you will report to the lab on 3 consecutive days for 2 submaximal exercise sessions on either an aquatic or land treadmill and an additional follow-up day with no exercise. Approximately 1 week later, this 3 day sequence will be repeated for the other exercise mode. Each visit will last approximately 1-2 hours.

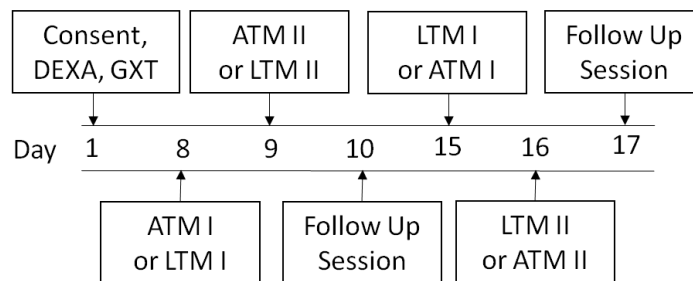


Figure 1. Study Timeline

All acute exercise sessions will include the following pre-exercise measures: fasted blood draw following 10 minutes of seated rest and measurements of flow mediated dilation (FMD), carotid-femoral pulse wave velocity (PWV), blood pressure, and heart rate following 10 minutes of supine rest. During acute exercise sessions, you will exercise at ~60% of maximal aerobic capacity for a duration required to expend 300

kcal. During exercise, oxygen consumption, heart rate, and blood pressure will be recorded. A blood sample will again be taken immediately post-exercise and 1-hour post exercise. Additionally, FMD and PWV will be measured 1-hour post exercise. Blood pressure will be taken automatically every 15 minutes during the 1-hour post-exercise recovery period. A summary of acute exercise session procedures is provided in Figure 2. These procedures will be repeated for the second day's session for a given exercise mode. The third follow up day, 24-hours following the last exercise session, will include only a blood draw, blood pressure, FMD, and PWV measurement. Ambulatory blood pressure will also be measured during the 24-hour period following each exercise session. This will require you to wear a mobile blood pressure device around your upper arm during the 24 hour period post-exercise that will periodically make an automatic blood pressure measurement and store the data. Following the initial three 3-day series, these procedures will be repeated approximately 7-days later for the alternate exercise mode. In addition to the above procedures, you will be asked to complete a 3-day food log on the two separate occasions during the exercise phases of the study.

| <u>Pre-Exercise</u> | <u>Exercise</u> | <u>Immediately Post-Exercise</u> | <u>1-Hour Post-Exercise</u> | <u>24-Hour Post-Exercise</u> |
|--|---|--|--|--|
| <ul style="list-style-type: none"> • Blood Draw • 10 min supine • BP • FMD/PWV | <ul style="list-style-type: none"> • 60% VO2max; 300 kcal • HR & RPE every 5 min • VO2 & BP every 10 min | <ul style="list-style-type: none"> • BP • Blood Draw | <ul style="list-style-type: none"> • 1 hour supine • Blood Draw • BP • FMD/PWV | <ul style="list-style-type: none"> • Blood Draw • 10 min supine • BP • FMD/PWV |

Figure 2. Acute Exercise Session Protocol

Blood samples will be made while fasted. All blood samples will be obtained from the antecubital vein using standard, sterile phlebotomy procedures by trained laboratory staff.

For the maximal GXT, you will walk or run on a land-based, motorized treadmill until you are exhausted. While you are doing this test, you will have electrodes attached to your chest to measure the activity of your heart through an electrocardiogram (ECG), you will breathe through a mouthpiece connected to a machine to measure the amount of oxygen your body is using, and your blood pressure will be measured. During the test, a licensed physician will be on site to review your ECG report to determine if there are any cardiovascular contraindications to exercise present. If there are any abnormalities shown to be present from your report, you will be given instructions on how to schedule further health screening tests elsewhere. Please note, that if you are not cleared by the physician during this testing day, you must be cleared by further cardiovascular testing elsewhere and provide clearance documentation from your doctor in order to be eligible to participate.

Your body bone density and body fat will be measured by lying at rest wearing exercise clothing in a DEXA (Dual Energy X-ray Absorptiometry) scanning machine. This machine will scan your body with a small amount of X-ray radiation. The radiation

exposure is comparatively less than the amount of natural radiation you would be subjected to flying in an airplane from Houston to Dallas. Anytime you feel uncomfortable in the machine you can remove yourself from it.

For resting PWV measures, you will lie quietly on a table for 10-15 minutes. You will be hooked up to an electrocardiogram to measure heart rate and the cardiac cycle. Following initial rest, the carotid, radial, and femoral arteries will be imaged separately with a noninvasive ultrasound probe. Images will be used to determine artery diameter, flow rate, pulse transit time, pulsatility index, and pulse wave velocity. Ultrasound imaging will take approximately 15 minutes. Carotid and radial artery sites can be easily imaged while clothed normally. It is necessary to wear loose fitting shorts and/or briefs for imaging of the femoral artery. Carotid-femoral pulse wave velocity is considered a better indicator of arterial stiffness than carotid-radial pulse wave velocity. These PWV measures will be made before exercise and 1-hour post-exercise.

For resting FMD measures, you will lie quietly on a table for 10-15 minutes. You will be hooked up to an electrocardiogram to measure heart rate and the cardiac cycle. Following initial rest, the brachial artery will be imaged with a noninvasive ultrasound probe. Following 10 minutes of rest, a blood pressure cuff will be inflated around your forearm to 200 mmHg for 5 minutes. Following cuff release, the vessel will continue to be imaged for 2-3 minutes. FMD measures will be made before exercise and 1-hour post-exercise.

Please note that at no time will any of the project research team or Texas A&M University be responsible for any medical costs outside of normal testing procedures during participation in the testing or training during this study.

What are the possible risks of this study?

1. *Maximal GXT*: The risks associated with the maximal effort GXT are comparable to those encountered during any strenuous physical activity. In otherwise healthy individuals who are properly screened for contraindications to exercise prior to the test and properly supervised by trained personnel during the test, the risk of potentially harmful or life-threatening events is extremely low. These include the risk of occasional abnormal blood pressure responses, syncope, heart dysrhythmia, severe dyspnea, and, in rare instances, heart attack. Mortality and morbidity rates for maximal exercise testing are reportedly 0.5 and 8.3, respectively per 10,000 tests. All subjects participating in this study will be asked to fill out a health history questionnaire to help assure the investigators there are no special risks to the subjects through participation in this study. Therefore, the risk of morbidity/mortality is considerably lower than 0.5 and 8.3 per 10,000. Throughout all testing procedures, the American College of Sports Medicine's *Guidelines for Exercise Testing and Prescription* (8th ed.) will be closely observed.

2. *Body composition assessment:* The DEXA method described for determination of body composition is an open system and uses very low intensity radiation for analysis. The machine will scan the body with a small amount of X-ray radiation. The radiation exposure is comparatively less than the amount of natural radiation one would be subjected to flying in an airplane from Houston to Dallas. Thus, the level of radiation the subject will be exposed to is below the levels that would constitute minimal risk to the subjects in this project.

3. *Blood sampling:* Obtaining the blood samples from the antecubital vein is a routine procedure in the Applied Exercise Science Laboratory and in many clinical settings. Adverse effects are very rare, although the puncture of the skin is accompanied by minor discomfort and may result in the development of a minor bruise next to the puncture site. As with any similar procedure disrupting the skin barrier, there is a risk of contracting an infection. This risk to the subjects (and to the technician) will be minimized through the use of accepted sterile procedures which include: (1) use of surgical rubber gloves by the technician; (2) antiseptic cleansing (70% alcohol) of the involved site prior to puncture; (3) use of sterile equipment and instruments for each sample; and (4) proper dressing of the wound with antiseptic and band-aid following sample collection. The amount of blood to be collected for each sample will be less than 5 ml from the antecubital vein and used blood plasma nitrite analysis. The AESL staff has had experience with over 2000 blood sampling procedures over many years with no known adverse consequences.

4. *Submaximal aerobic exercise sessions and exercise training:* Again, as with the maximal exercise test, there exists a risk of abnormal blood pressure response, syncope, heart dysrhythmia, severe dyspnea, and, in rare instances, heart attack during exercise. However, these risks will be minimized since the subjects in this study will be previously screened for contraindications for heavy exercise through maximal exercise testing. In addition, with regular exercise training there is a risk of an injury to joints or muscles, such as ankle, knee, or hip sprains or, rarely, fractures, and muscle strains. Every reasonable precaution will be taken to ensure that the exercise is carried out in a safe manner. Exercise heart rates and blood pressures will be checked and charted regularly by trained exercise technicians and all exercise prescription procedures will conform to the *Guidelines for Exercise Testing and Prescription* (8th ed.) published by the American College of Sports Medicine.

5. *Flow Mediated Dilation/Pulse Wave Velocity:* During the FMD procedure, the subject's hand might become slightly numb during the 5 minutes of blood pressure cuff inflation around the forearm. This slight tingling is normal and resolves quickly following cuff release. Otherwise there are no known risks from the non-invasive vascular measurements via ultrasound.

What are the possible benefits of this study?

The preliminary screening will provide valuable information to each subject regarding their relative risk for cardiovascular disease (CVD) and present physical fitness status. Furthermore, blood pressure and ECG will be monitored during the max GXT; this will provide each subject with important information related to the functional status of the cardiovascular system during maximal exertion, and will provide other ancillary information about the health of the cardiovascular system. Additionally, subjects will be provided with their individual results regarding brachial artery flow mediated dilation (endothelial function) and pulse wave velocity (arterial stiffness), which are markers of cardiovascular health. The body composition assessment will provide each subject with information regarding current bone mass, ideal body weight for their age and gender, and the amount of fat that may be reasonably and safely lost. From the dietary information provided, each subject will learn about the composition of their current eating habits, and will be able to compare this to the composition of diets recommended to promote health. Any individual with abnormalities noted on any of the preliminary screenings or testing procedures will be advised to consult their private physician for further evaluation.

Do I have to participate?

No. Your participation is voluntary. You may decide not to participate or to withdraw at any time without your current or future relations with Texas A&M University or the Applied Exercise Science Laboratory being affected.

Will I be compensated?

There will be no monetary compensation.

Who will know about my participation in this research study?

The data collected during this study is confidential and the names of all the subjects will be entered as a code in data analysis to ensure the confidentiality. The records of this study will be kept private. No identifiers linking you to the study will be included in any sort of report that might be published. Research records will be stored securely, and only Dr. Stephen F. Crouse and his research collaborators will have access to the records.

Your decision whether or not to participate will not affect your current relations with Texas A&M University. If you decide to participate, you are free to refuse any situations that may be objectionable. You can withdraw at any time without your relations with the university, job, benefits, etc., being affected.

Contact Information

Whom do I contact with questions about the research?

If you have questions regarding this study, you may contact:

Dustin Joubert
Office: (979) 845-9418
Email: DJoubert@hlkn.tamu.edu

And/or

The Applied Exercise Science Laboratory
Phone: (979) 845-9418
Fax: (979) 862-2207

Whom do I contact about my rights as a research participant?

This research study has been reviewed by the Human Subjects' Protection Program and/or the Institutional Review Board at Texas A&M University. For research-related problems or questions regarding your rights as a research participant, you can contact these offices at (979)458-4067 or irb@tamu.edu.

Signature

Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in this study.

Signature of Participant: _____ **Date:** _____

Printed Name: _____

Signature of Person Obtaining Consent: _____ **Date:** _____

Printed Name: _____

APPENDIX B

THREE DAY DIET RECORD

Name:_____ Age:_____ Ht:_____ Wt:_____

DIRECTIONS: This Three Day Diet Record is designed to measure your food intake over the course of three consecutive days. Please make sure that ONE recorded day is a weekend. Because of this requirement, this record should be filled out **Thursday, Friday, Saturday OR Sunday, Monday, Tuesday**.

1. Records should be kept over a time period that best represents your “normal” eating patterns for 2 weekdays and one weekend day. For example, if Monday is a work holiday, it is unlikely that you will eat as you normally would.
2. Record **ALL** food and drink (**including water**) that you consume on each day. Record both the type of food or drink and the amounts consumed.
3. Please be as specific on foods and amounts as possible. For example, if you eat a turkey sandwich, please record the type of bread (white, whole wheat, rye, etc), number of slices of meat, and any additional items (cheese, tomato, mayonnaise, etc). Also include brand names of items when possible. For help in determining what is considered a serving, see the serving size chart on page 2 for some common food items.
4. Page 3 shows a sample day of the diet record. Please read this to help you become familiar with the recording format.
5. If you have any questions about filling out the record, please contact laboratory staff for assistance.
6. Return this record to the laboratory staff once it is complete.

Please do not change your diet in any way during the course of the study. Maintain normal eating habits, please do not begin a “diet”. If you travel, don’t worry, these changes from normal are only temporary.

Serving Size Chart



1 Cup cereal flakes or 1 baked potato = size of a fist



$\frac{1}{2}$ cup cooked rice, pasta or potato = size of an ice cream scoop



1 pancake = size of a CD



1 cup of salad greens or 1 medium fruit = size of a baseball



$\frac{1}{2}$ cup fresh fruit or vegetables = size of a standard light bulb



$\frac{1}{4}$ cup dried fruit = 1 large egg



3 oz. meat, fish, poultry = size of a deck of cards



2 Tbsp peanut butter = size of a golf ball



1 $\frac{1}{2}$ oz. cheese = 4 stacked dice or 2 cheese slices; 1 tsp margarine, butter and spreads = 1 dice

Day 1

Food Eaten

Serving Size

[illegible]

Day 2

Food Eaten

Serving Size

[illegible]

Day 3 Food Eaten

Serving Size

[illegible]

**Sample
Food Eaten**

Serving Size

| | | | |
|--------------------------------|-------------|--|--|
| Breakfast | | | |
| coffee (caffinated) | 1-8oz cup | | |
| w/ half&half | 2 Tbsp | | |
| w/ Splenda | 1 Tbsp | | |
| Raisin Bran cereal | 1 cup | | |
| w/ 1% milk | 1 cup | | |
| Multivitamin | 1 vitamin | | |
| Lunch | | | |
| Turkey sandwich (homemade) | | | |
| w/ turkey deli meat | 3 slices | | |
| w/ Kraft American cheese | 1 slice | | |
| w/ Lite mayo | 2 Tbsp | | |
| w/ whole wheat bread | 2 slices | | |
| w/ mustard | 1 tbsp | | |
| apple | 1 medium | | |
| Lay's potato chips | 1 snack bag | | |
| Sprite | 12oz can | | |
| Snacks | | | |
| water | 20oz bottle | | |
| Nature's Own honey granola bar | 2 bars | | |
| Hershey's Kisses | 3 kisses | | |
| Lemon-lime Gatorade | 32oz bottle | | |
| Dinner | | | |
| McDonald's Big Mac | | | |
| w/ cheese and mayo | | | |
| french fries | medium | | |
| Diet Coke | medium | | |
| Bluebell Vanilla Ice Cream | 2 scoops | | |

Diet Compliance Form

DIET COMPLIANCE

NAME: _____

DATE: _____

1. My diet (**has / has not**) changed from the last diet record submitted.
2. My diet changed as follows:

[illegible]

Printed Name_____

Signature _____

APPENDIX C

Acute Exercise Session Data Sheet

Name: _____

Date: _____ Time: _____

Age: _____ Height: _____ Weight: _____

1. Have you exercised today? Y or N
 2. Have you consumed any caffeinated products today? Y or N
 3. Have you consumed any alcoholic beverages today? Y or N
 4. Have you consumed any products containing nicotine today? Y or N
 5. Current medication or supplements? _____
 6. When was your last meal? _____
-

Following 10 Minutes of seated rest, blood pressure should be taken on the left arm while the subject is seated with their feet uncrossed and flat on the floor. Blood pressure will be taken three times at 3-minute intervals via automated blood pressure measurement.

| | SBP (mmHg) | DBP (mmHg) | MAP (mmHG) | HR (bpm) |
|---|------------|------------|------------|----------|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |

Post-Exercise Measures

| | SBP (mmHg) | DBP (mmHg) | MAP (mmHG) | HR (bpm) |
|----|------------|------------|------------|----------|
| 10 | | | | |
| 20 | | | | |
| 30 | | | | |
| 40 | | | | |
| 50 | | | | |
| 60 | | | | |

APPENDIX D

Statistical Analysis and Raw Data

Statistical Design

A 2-way (mode x day/time point) repeated measures ANOVA was the primary model of statistical analysis. The specific analysis used for each dependent variable were as follows: resting blood pressure – 2 (Mode: ATM vs. LTM) x 3 (Day: 1, 2, 3F), post-exercise blood pressure change and ambulatory blood pressure – 2 (Mode) x 2 (Day), FMD and PWV – 2 (Mode) x 5 (Time point: 1 Pre, 1 Post, 2 Pre, 2 Post, 3F), change in FMD and PWV – 2 (Mode) x 3 (Time point: 1, 24, 48 hours post-exercise), plasma nitrates/nitrites – 2 (Mode) x 7 (Time point: 1 Pre, 1 IPE, 1 1hr, 2 Pre, 2 IPE, 2 1hr, 3F), plasma volume change – 2 (Mode) x 6 (Time point: 1 IPE, 1 1hr, 2 Pre, 2 IPE, 2 1hr, 3F). Statistical analysis were performed in IBM SPSS Statistics 23 (IBM, New York).

SPSS Procedures

- Analyze > General Linear Model > Repeated Measures
- Within Subjects Factor Name: Mode > 2 levels; Add
- Within Subjects Factor Name: Day/Time point > # levels based on variable; Add
- Click “define” once factors are added
- Select dependent variables and add to within subject factors making sure each variable coincides with defined factor name and level
- Select “options”. Highlight factors and add to “display means for”...check “compare main effects” and select LSD. Check “descriptive statistics”,

“estimates of effect size”, “observed power”, and “parameter estimates”. Click Continue.

- Click OK to run analysis.

Data Abbreviations

| | |
|-----------|--|
| Subject | Identifies subject by ID code |
| Order | 1 = ATM done first in sequence, 2 = LTM done first in sequence |
| Age | Age in years |
| Race | Race of subject |
| Height | Height of subject in inches |
| Weight | Weight of subject in lbs |
| BruceTime | Minutes completed during Bruce max treadmill test |
| VO2 (rel) | Relative VO2max (ml/kg/min) |
| VO2 (abs) | Absolute VO2max (l/min) |
| %Fat | DEXA %Body Fat |
| And%Fat | Android %Fat |
| Gyn%Fat | Gynoid %Fat |
| Mass (kg) | DEXA body mass in kg |
| FatMass | DEXA fat mass in kg |
| LeanMass | DEXA lean mass in kg |
| Scr | Screening |
| SBP | Systolic blood pressure in mmHg |
| DBP | Diastolic blood pressure in mmHg |
| MAP | Mean arterial pressure in mmHg |
| PP | Pulse Pressure in mmHg |
| HR | Heart rate |
| ATM | Aquatic treadmill |
| LTM | Land treadmill |
| A1 | ATM exercise day 1 |
| A2 | ATM exercise day 2 |
| AF | ATM day 3 follow-up |
| L1 | LTM exercise day 1 |
| L2 | LTM exercise day 2 |
| LF | LTM day 3 follow-up |
| Pre | Pre-exercise |
| IPE | Immediately post-exercise |
| 1hr | 1-hour post-exercise |
| 24hr | 24-hour post-exercise |
| 48hr | 48-hour post-exercise |
| SBPΔ | Change in SBP post-exercise (pre-post) |

| | |
|-------|---|
| DBPΔ | Change in DBP post-exercise (pre-post) |
| MAPΔ | Change in MAP post-exercise (pre-post) |
| SBPA | Ambulatory SBP – Awake |
| DBPA | Ambulatory DBP – Awake |
| MAPA | Ambulatory MAP – Awake |
| PPA | Ambulatory PP – Awake |
| HRA | Ambulatory HR – Awake |
| FMD | Flow-mediated dilation in %dilation from baseline |
| FlowΔ | Change in brachial flow post occlusion (pre-post) in cm/s |
| PWV | Pulse wave velocity in m/s |
| PV | Plasma volume calculated as %change from baseline |
| NO | Plasma nitrates/nitrites in μM adjusted for PV changes |

Raw Data

| Subject | Order | Age | Race | Height | Weight | BruceTime |
|---------|-----------|-----------|-----------|---------|---------|-----------|
| AC22 | 2 | 22 | Hispanic | 71.0 | 205 | 11.83 |
| CB59 | 1 | 59 | Caucasian | 69.0 | 167 | 7.10 |
| DB21 | 2 | 21 | Caucasian | 69.0 | 141 | 14.00 |
| DC26 | 1 | 26 | Caucasian | 73.0 | 174 | 12.15 |
| DH24 | 1 | 24 | Caucasian | 70.0 | 269 | 9.55 |
| JG28 | 2 | 28 | Hispanic | 72.0 | 153 | 12.17 |
| JH20 | 1 | 20 | Caucasian | 68.0 | 153 | 13.75 |
| JL43 | 1 | 43 | Caucasian | 76.0 | 246 | 9.57 |
| KD44 | 1 | 44 | Caucasian | 74.0 | 230 | 9.50 |
| KK22 | 2 | 22 | Asian | 68.0 | 175 | 12.13 |
| KS38 | 1 | 38 | Asian | 66.0 | 156 | 11.33 |
| LD34 | 2 | 34 | Caucasian | 71.0 | 205 | 10.58 |
| MA19 | 1 | 19 | Asian | 72.0 | 178 | 10.42 |
| PP21 | 2 | 21 | Caucasian | 73.5 | 181 | 13.47 |
| SJ52 | 2 | 52 | Caucasian | 68.0 | 183 | 9.10 |
| SL30 | 2 | 30 | Black | 74.0 | 372 | 8.00 |
| SL46 | 2 | 46 | Caucasian | 67.0 | 223 | 9.33 |
| WF36 | 1 | 36 | Caucasian | 73.0 | 228 | 9.58 |
| ZK23 | 1 | 23 | Caucasian | 69.0 | 202 | 10.30 |
| Subject | VO2 (rel) | VO2 (abs) | %Fat | And%Fat | Gyn%Fat | Mass (kg) |
| AC22 | 42.7 | 4.010 | 32.0 | 43.1 | 38.1 | 89.0 |
| CB59 | 23.3 | 1.765 | 28.9 | 44.0 | 34.5 | 74.7 |
| DB21 | 53.6 | 3.427 | 8.0 | 12.0 | 13.1 | 64.8 |
| DC26 | 43.6 | 3.441 | 22.4 | 33.6 | 29.1 | 78.7 |
| DH24 | 32.7 | 3.990 | 42.0 | 55.5 | 39.1 | 122.2 |
| JG28 | 43.2 | 2.998 | 24.8 | 38.3 | 32.2 | 68.9 |

| | | | | | | |
|------|------|-------|------|------|------|-------|
| JH20 | 49.1 | 3.407 | 12.9 | 17.5 | 16.9 | 67.2 |
| JL43 | 31.8 | 3.547 | 33.6 | 43.7 | 45.0 | 108.1 |
| KD44 | 37.7 | 4.030 | 32.7 | 43.0 | 37.0 | 99.9 |
| KK22 | 42.7 | 3.388 | 33.5 | 44.7 | 38.8 | 79.8 |
| KS38 | 40.2 | 2.844 | 24.0 | 33.9 | 31.0 | 70.7 |
| LD34 | 37.2 | 3.530 | 28.8 | 43.8 | 29.7 | 91.1 |
| MA19 | 43.1 | 3.481 | 30.1 | 38.6 | 38.5 | 80.9 |
| PP21 | 49.0 | 4.030 | 13.8 | 22.8 | 17.8 | 82.5 |
| SJ52 | 38.9 | 3.230 | 34.8 | 46.7 | 41.0 | 83.3 |
| SL30 | 23.3 | 3.894 | 44.7 | 57.8 | 46.7 | 156.4 |
| SL46 | 29.0 | 2.970 | 35.8 | 48.3 | 42.6 | 98.8 |
| WF36 | 32.4 | 3.343 | 40.1 | 53.1 | 41.7 | 102.1 |
| ZK23 | 33.1 | 3.030 | 36.8 | 44.0 | 43.3 | 87.3 |

| Subject | FatMass | LeanMass | ScrSBP | ScrDBP | ScrMAP | ScrHR |
|---------|---------|----------|--------|--------|--------|-------|
| AC22 | 29.5 | 59.5 | 140 | 89 | 107 | 94 |
| CB59 | 21.6 | 50.1 | 137 | 90 | 106 | 72 |
| DB21 | 5.2 | 56.9 | 125 | 78 | 94 | 58 |
| DC26 | 17.6 | 58.0 | 120 | 75 | 91 | 59 |
| DH24 | 51.3 | 67.3 | 143 | 79 | 100 | 91 |
| JG28 | 17.1 | 49.2 | 121 | 67 | 85 | 85 |
| JH20 | 9.0 | 58.2 | 128 | 66 | 90 | 83 |
| JL43 | 37.7 | 70.5 | 141 | 88 | 107 | 66 |
| KD44 | 34.1 | 65.9 | 127 | 89 | 101 | 60 |
| KK22 | 26.5 | 49.1 | 125 | 68 | 89 | 72 |
| KS38 | 17.0 | 50.8 | 137 | 79 | 98 | 66 |
| LD34 | 27.4 | 63.7 | 131 | 78 | 97 | 81 |
| MA19 | 24.3 | 53.5 | 131 | 69 | 92 | 95 |
| PP21 | 11.4 | 67.5 | 121 | 75 | 91 | 70 |
| SJ52 | 29.0 | 50.9 | 127 | 84 | 99 | 53 |
| SL30 | 71.5 | 84.9 | 131 | 70 | 92 | 69 |
| SL46 | 36.6 | 62.2 | 125 | 76 | 93 | 66 |
| WF36 | 41.0 | 57.2 | 139 | 85 | 104 | 76 |
| ZK23 | 33.6 | 53.7 | 128 | 77 | 95 | 109 |

| Subject | A1PreSBP | A2PreSBP | AFPreSBP | L1PreSBP | L2PreSBP | LFPreSBP |
|---------|----------|----------|----------|----------|----------|----------|
| AC22 | 119 | 136 | 125 | 125 | 126 | 122 |
| CB59 | 122 | 125 | 132 | 125 | 129 | 115 |
| DB21 | 114 | 123 | 120 | 109 | 111 | 110 |
| DC26 | 122 | 123 | 116 | 120 | 109 | 124 |
| DH24 | 126 | 109 | 119 | 115 | 109 | 119 |
| JG28 | 114 | 108 | 112 | 109 | 111 | 109 |

| | | | | | | |
|------|-----|-----|-----|-----|-----|-----|
| JH20 | 123 | 126 | 123 | 118 | 117 | 122 |
| JL43 | 148 | 146 | 133 | 136 | 135 | 132 |
| KD44 | 118 | 117 | 110 | 119 | 124 | 120 |
| KK22 | 120 | 117 | 123 | 134 | 127 | 125 |
| KS38 | 129 | 121 | 120 | 116 | 126 | 114 |
| LD34 | 115 | 124 | 131 | 120 | 124 | 118 |
| MA19 | 117 | 110 | 112 | 101 | 104 | 108 |
| PP21 | 116 | 109 | 111 | 116 | 106 | 114 |
| SJ52 | 118 | 120 | 117 | 124 | 115 | 122 |
| SL30 | 141 | 129 | 129 | 139 | 125 | 127 |
| SL46 | 121 | 121 | 117 | 132 | 125 | 120 |
| WF36 | 127 | 127 | 116 | 121 | 118 | 121 |
| ZK23 | 124 | 118 | 119 | 115 | 110 | 108 |

| Subject | A1PreDBP | A2PreDBP | AFPreDBP | L1PreDBP | L2PreDBP | LFPreDBP |
|---------|----------|----------|----------|----------|----------|----------|
| AC22 | 82 | 80 | 73 | 77 | 79 | 70 |
| CB59 | 79 | 78 | 87 | 80 | 79 | 77 |
| DB21 | 66 | 66 | 74 | 63 | 62 | 66 |
| DC26 | 75 | 74 | 68 | 69 | 65 | 73 |
| DH24 | 71 | 67 | 69 | 69 | 65 | 67 |
| JG28 | 70 | 68 | 69 | 67 | 68 | 70 |
| JH20 | 69 | 68 | 67 | 66 | 62 | 65 |
| JL43 | 89 | 81 | 78 | 82 | 83 | 71 |
| KD44 | 81 | 79 | 79 | 75 | 83 | 79 |
| KK22 | 62 | 63 | 65 | 65 | 64 | 65 |
| KS38 | 82 | 69 | 68 | 66 | 73 | 65 |
| LD34 | 75 | 73 | 75 | 75 | 69 | 68 |
| MA19 | 60 | 63 | 64 | 59 | 57 | 59 |
| PP21 | 70 | 66 | 62 | 67 | 65 | 66 |
| SJ52 | 73 | 74 | 69 | 77 | 70 | 68 |
| SL30 | 82 | 78 | 81 | 81 | 74 | 77 |
| SL46 | 86 | 85 | 90 | 93 | 93 | 85 |
| WF36 | 89 | 88 | 81 | 86 | 84 | 87 |
| ZK23 | 75 | 69 | 78 | 76 | 69 | 63 |

| Subject | A1PreMAP | A2PreMAP | AFPreMAP | L1PreMAP | L2PreMAP | LFPreMAP |
|---------|----------|----------|----------|----------|----------|----------|
| AC22 | 95 | 100 | 93 | 92 | 96 | 90 |
| CB59 | 94 | 94 | 102 | 96 | 95 | 89 |
| DB21 | 86 | 88 | 89 | 83 | 83 | 85 |
| DC26 | 91 | 92 | 87 | 89 | 84 | 91 |
| DH24 | 91 | 85 | 87 | 87 | 84 | 88 |
| JG28 | 87 | 84 | 86 | 85 | 86 | 86 |

| | | | | | | |
|------|-----|-----|----|-----|-----|----|
| JH20 | 90 | 90 | 89 | 86 | 85 | 87 |
| JL43 | 111 | 105 | 97 | 95 | 102 | 92 |
| KD44 | 94 | 90 | 88 | 90 | 97 | 93 |
| KK22 | 86 | 85 | 88 | 91 | 89 | 89 |
| KS38 | 98 | 89 | 88 | 86 | 92 | 85 |
| LD34 | 89 | 91 | 93 | 90 | 90 | 88 |
| MA19 | 84 | 84 | 84 | 78 | 79 | 81 |
| PP21 | 88 | 85 | 83 | 88 | 83 | 84 |
| SJ52 | 89 | 90 | 90 | 93 | 88 | 89 |
| SL30 | 102 | 96 | 97 | 101 | 92 | 96 |
| SL46 | 98 | 96 | 98 | 105 | 103 | 97 |
| WF36 | 102 | 101 | 92 | 96 | 95 | 96 |
| ZK23 | 90 | 88 | 92 | 90 | 86 | 83 |

| Subject | A1PreHR | A2PreHR | AFPreHR | L1PreHR | L2PreHR | LFPreHR |
|---------|---------|---------|---------|---------|---------|---------|
| AC22 | 81 | 69 | 79 | 80 | 64 | 77 |
| CB59 | 63 | 62 | 65 | 69 | 68 | 65 |
| DB21 | 50 | 56 | 55 | 51 | 56 | 56 |
| DC26 | 68 | 71 | 76 | 65 | 66 | 67 |
| DH24 | 91 | 88 | 82 | 79 | 81 | 73 |
| JG28 | 64 | 62 | 82 | 69 | 59 | 68 |
| JH20 | 79 | 82 | 77 | 71 | 81 | 86 |
| JL43 | 82 | 82 | 114 | 78 | 71 | 90 |
| KD44 | 64 | 61 | 61 | 69 | 61 | 60 |
| KK22 | 73 | 61 | 73 | 72 | 75 | 70 |
| KS38 | 69 | 57 | 53 | 57 | 56 | 58 |
| LD34 | 86 | 79 | 81 | 80 | 82 | 82 |
| MA19 | 67 | 75 | 69 | 67 | 63 | 64 |
| PP21 | 67 | 68 | 68 | 67 | 66 | 68 |
| SJ52 | 48 | 47 | 52 | 49 | 45 | 49 |
| SL30 | 68 | 62 | 58 | 71 | 61 | 59 |
| SL46 | 71 | 69 | 73 | 68 | 75 | 97 |
| WF36 | 71 | 74 | 72 | 69 | 76 | 72 |
| ZK23 | 68 | 78 | 70 | 83 | 59 | 73 |

| Subject | A1SBPA | A2SBPA | L1SBPA | L2SBPA |
|---------|--------|--------|--------|--------|
| AC22 | 8 | 5 | -1 | -6 |
| CB59 | 2 | -3 | 19 | -7 |
| DB21 | -7 | -5 | 0 | -4 |
| DC26 | -16 | -15 | -6 | 4 |
| DH24 | -10 | 5 | -2 | 2 |
| JG28 | -6 | -5 | -2 | -3 |

| | | | | |
|------|-----|-----|-----|----|
| JH20 | -14 | -17 | -7 | -2 |
| JL43 | -1 | -10 | -8 | -9 |
| KD44 | -1 | 2 | -2 | -6 |
| KK22 | 0 | 7 | -5 | -3 |
| KS38 | -7 | -1 | 5 | -4 |
| LD34 | 12 | -2 | -1 | -7 |
| MA19 | -9 | 1 | 8 | -2 |
| PP21 | 8 | 5 | 3 | 7 |
| SJ52 | 0 | -2 | -3 | 7 |
| SL30 | -6 | 5 | -8 | 5 |
| SL46 | 3 | 4 | -13 | 4 |
| WF36 | -8 | -6 | -4 | -2 |
| ZK23 | -9 | 1 | -3 | 1 |

| Subject | A1DBPΔ | A2DBPΔ | L1DBPΔ | L2DBPΔ |
|---------|--------|--------|--------|--------|
| AC22 | -2 | 7 | 0 | -3 |
| CB59 | 5 | 1 | 10 | -1 |
| DB21 | -3 | 3 | -4 | 2 |
| DC26 | -7 | -6 | 1 | 2 |
| DH24 | 0 | 2 | -1 | 2 |
| JG28 | -3 | -2 | -1 | 1 |
| JH20 | -5 | -5 | -1 | 3 |
| JL43 | -3 | 4 | 2 | -6 |
| KD44 | 0 | 2 | 8 | -4 |
| KK22 | 5 | 0 | 4 | -3 |
| KS38 | -12 | 0 | 5 | 0 |
| LD34 | -2 | -1 | 2 | 4 |
| MA19 | 3 | 3 | 0 | -1 |
| PP21 | 4 | 2 | 4 | 3 |
| SJ52 | 1 | -3 | -1 | 2 |
| SL30 | 6 | 8 | 2 | 5 |
| SL46 | 3 | 2 | -2 | -3 |
| WF36 | -8 | -8 | -10 | -3 |
| ZK23 | 3 | 3 | -4 | -1 |

| Subject | A1MAPΔ | A2MAPΔ | L1MAPΔ | L2MAPΔ |
|---------|--------|--------|--------|--------|
| AC22 | 3 | 7 | 1 | -4 |
| CB59 | 3 | -1 | 13 | -2 |
| DB21 | -4 | 0 | -2 | -1 |
| DC26 | -7 | -6 | -2 | 2 |
| DH24 | -3 | 1 | -2 | 1 |
| JG28 | -3 | -2 | -1 | -1 |

| | | | | |
|------|----|----|----|----|
| JH20 | -6 | -8 | -2 | 0 |
| JL43 | -4 | -2 | 3 | -8 |
| KD44 | -2 | 3 | 5 | -5 |
| KK22 | 2 | 2 | 0 | -2 |
| KS38 | -9 | -1 | 3 | -2 |
| LD34 | 3 | -1 | 0 | -1 |
| MA19 | -1 | 0 | 2 | -2 |
| PP21 | 3 | 1 | 2 | 3 |
| SJ52 | 1 | -1 | -1 | 3 |
| SL30 | 3 | 5 | -2 | 4 |
| SL46 | 2 | 1 | -6 | -1 |
| WF36 | -9 | -7 | -7 | -3 |
| ZK23 | 0 | 1 | -3 | -1 |

| Subject | A1HRA | A2HRA | L1HRA | L2HRA |
|---------|-------|-------|-------|-------|
| AC22 | -3 | 18 | -2 | 16 |
| CB59 | 12 | 7 | 6 | 4 |
| DB21 | 24 | 13 | 20 | 19 |
| DC26 | 21 | 12 | 9 | 15 |
| DH24 | 2 | 5 | 0 | -3 |
| JG28 | 15 | 14 | 12 | 22 |
| JH20 | -4 | -4 | 16 | -4 |
| JL43 | 6 | 7 | 18 | 26 |
| KD44 | 9 | 7 | 2 | 4 |
| KK22 | -1 | 13 | 8 | 14 |
| KS38 | 7 | 7 | 8 | 11 |
| LD34 | 14 | 21 | 9 | 8 |
| MA19 | 27 | 14 | 14 | 8 |
| PP21 | 9 | 6 | 14 | 9 |
| SJ52 | 6 | 8 | 3 | 8 |
| SL30 | 5 | 6 | 0 | 3 |
| SL46 | 8 | 6 | 14 | 4 |
| WF36 | 19 | 12 | 10 | 7 |
| ZK23 | 22 | 1 | 6 | 15 |

| Subject | A1SBPA | A2SBPA | L1SBPA | L2SBPA | A1DBPA | A2DBPA |
|---------|--------|--------|--------|--------|--------|--------|
| AC22 | 154 | 144 | 147 | 136 | 92 | 84 |
| CB59 | 149 | 159 | 173 | 144 | 87 | 91 |
| DB21 | 150 | 169 | 130 | 156 | 73 | 80 |
| DC26 | 130 | 129 | 135 | 131 | 69 | 67 |
| DH24 | 130 | 117 | 137 | 124 | 76 | 64 |
| JG28 | 140 | 136 | 134 | 141 | 76 | 74 |

| | | | | | | |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|
| JH20 | 138 | 146 | 140 | 143 | 78 | 88 |
| JL43 | 153 | 148 | 146 | 131 | 95 | 92 |
| KD44 | 130 | 133 | 135 | 138 | 82 | 78 |
| KK22 | 126 | 124 | 132 | 134 | 65 | 64 |
| KS38 | 138 | 142 | 134 | 133 | 79 | 81 |
| LD34 | 140 | 134 | 140 | 137 | 83 | 76 |
| MA19 | 126 | 120 | 115 | 122 | 68 | 64 |
| PP21 | 128 | 122 | 123 | 122 | 73 | 64 |
| SJ52 | 142 | 145 | 126 | 126 | 88 | 83 |
| SL46 | 137 | 136 | 139 | 141 | 84 | 88 |
| WF36 | 132 | 133 | 138 | 135 | 83 | 84 |
| ZK23 | 133 | 122 | 122 | 123 | 78 | 71 |
| Subject | L1DBPA | L2DBPA | A1MAPA | A2MAPA | L1MAPA | L2MAPA |
| AC22 | 88 | 78 | 113 | 104 | 108 | 97 |
| CB59 | 97 | 83 | 108 | 114 | 122 | 103 |
| DB21 | 69 | 79 | 99 | 110 | 89 | 105 |
| DC26 | 72 | 69 | 89 | 88 | 93 | 90 |
| DH24 | 75 | 72 | 94 | 82 | 96 | 89 |
| JG28 | 73 | 74 | 97 | 95 | 93 | 96 |
| JH20 | 85 | 81 | 98 | 107 | 103 | 102 |
| JL43 | 96 | 79 | 114 | 111 | 113 | 96 |
| KD44 | 83 | 83 | 98 | 96 | 100 | 101 |
| KK22 | 72 | 74 | 85 | 84 | 92 | 94 |
| KS38 | 78 | 78 | 99 | 101 | 97 | 96 |
| LD34 | 82 | 83 | 102 | 95 | 101 | 101 |
| MA19 | 56 | 64 | 87 | 83 | 76 | 83 |
| PP21 | 70 | 69 | 91 | 83 | 88 | 87 |
| SJ52 | 72 | 72 | 106 | 104 | 90 | 90 |
| SL46 | 92 | 87 | 102 | 104 | 108 | 105 |
| WF36 | 87 | 83 | 99 | 100 | 104 | 100 |
| ZK23 | 76 | 76 | 96 | 88 | 91 | 92 |
| Subject | A1PPA | A2PPA | L1PPA | L2PPA | A1HRA | A2HRA |
| AC22 | 62 | 60 | 59 | 58 | 78 | 82 |
| CB59 | 62 | 68 | 76 | 61 | 74 | 73 |
| DB21 | 77 | 89 | 61 | 77 | 67 | 73 |
| DC26 | 61 | 62 | 63 | 62 | 68 | 70 |
| DH24 | 54 | 53 | 62 | 52 | 82 | 80 |
| JG28 | 64 | 62 | 61 | 67 | 78 | 74 |
| JH20 | 60 | 58 | 55 | 62 | 79 | 92 |
| JL43 | 58 | 56 | 50 | 52 | 89 | 101 |

| | | | | | | |
|------|----|----|----|----|----|----|
| KD44 | 48 | 55 | 52 | 55 | 68 | 72 |
| KK22 | 61 | 60 | 60 | 60 | 66 | 69 |
| KS38 | 59 | 61 | 56 | 55 | 65 | 64 |
| LD34 | 57 | 58 | 58 | 54 | 95 | 91 |
| MA19 | 58 | 56 | 59 | 58 | 82 | 74 |
| PP21 | 55 | 58 | 53 | 53 | 71 | 68 |
| SJ52 | 54 | 62 | 54 | 54 | 58 | 56 |
| SL46 | 53 | 48 | 47 | 54 | 84 | 81 |
| WF36 | 49 | 49 | 51 | 52 | 80 | 74 |
| ZK23 | 55 | 51 | 46 | 47 | 80 | 69 |

| Subject | L1HRA | L2HRA |
|----------------|--------------|--------------|
| AC22 | 82 | 74 |
| CB59 | 76 | 77 |
| DB21 | 67 | 67 |
| DC26 | 69 | 75 |
| DH24 | 78 | 72 |
| JG28 | 75 | 77 |
| JH20 | 88 | 85 |
| JL43 | 91 | 78 |
| KD44 | 66 | 65 |
| KK22 | 75 | 88 |
| KS38 | 62 | 66 |
| LD34 | 91 | 87 |
| MA19 | 72 | 75 |
| PP21 | 71 | 72 |
| SJ52 | 53 | 56 |
| SL46 | 82 | 90 |
| WF36 | 73 | 70 |
| ZK23 | 86 | 71 |

| Subject | A1PRE FMD | A1POST FMD | A2PRE FMD | A2POST FMD | AF FMD | L1PRE FMD |
|----------------|----------------------|-----------------------|----------------------|-----------------------|-------------------|----------------------|
| AC22 | 15.92 | 18.86 | 10.00 | 7.55 | 11.96 | 12.59 |
| CB59 | 4.68 | 3.87 | 4.42 | 2.72 | 5.88 | 4.29 |
| DB21 | 1.37 | 5.10 | 0.80 | 5.67 | -0.55 | 2.53 |
| DC26 | 7.02 | 4.62 | 1.17 | 6.46 | 3.50 | 6.07 |
| DH24 | 5.93 | 7.44 | 16.09 | 8.23 | 10.87 | 8.60 |
| JG28 | 7.07 | 4.69 | 6.25 | -0.47 | 1.18 | 5.17 |
| JH20 | 5.29 | 3.57 | 11.11 | 2.52 | 1.90 | 11.49 |
| JL43 | 7.02 | 4.76 | 7.25 | 7.82 | 7.32 | 5.71 |
| KD44 | 2.71 | 1.90 | 4.56 | 0.58 | 5.06 | 1.37 |

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|----------------|----------------|---------------|----------------|----------------|---------------|----------------|-------|
| | KK22 | 3.36 | 8.31 | 5.13 | 5.64 | 4.02 | 5.15 |
| | LD34 | 7.52 | 6.65 | 4.12 | 8.10 | 7.87 | 4.88 |
| | MA19 | 5.19 | 10.31 | 4.49 | 5.38 | 8.05 | 5.19 |
| | PP21 | 5.34 | 10.98 | 6.73 | 13.58 | 3.11 | 0.70 |
| | SJ52 | 5.28 | 6.57 | 6.81 | 6.36 | 18.04 | 9.81 |
| | SK38 | 6.85 | 8.38 | 13.26 | 5.81 | 6.96 | 5.13 |
| | SL30 | 13.72 | 15.03 | 12.53 | 8.96 | 12.02 | 8.58 |
| | SL46 | 0.62 | 4.51 | 14.29 | 8.13 | 8.37 | 8.62 |
| | WF36 | 6.83 | 1.24 | 4.90 | 2.15 | 5.63 | 8.09 |
| | ZK23 | 12.47 | 14.60 | 5.87 | 11.95 | 12.56 | 12.67 |
| Subject | L1Post | L2PRE | L2POST | LF | A1 1HR | A1 24HR | |
| | FMD | FMD | FMD | FMD | FMDΔ | FMDΔ | |
| | AC22 | 10.34 | 10.77 | 6.64 | 12.47 | 2.94 | -5.92 |
| | CB59 | 0.24 | 4.29 | 3.82 | 2.76 | -0.81 | -0.26 |
| | DB21 | 3.31 | 2.75 | 2.83 | -1.37 | 3.73 | -0.57 |
| | DC26 | 5.88 | 8.94 | 5.20 | 4.92 | -2.40 | -5.85 |
| | DH24 | 8.84 | 8.92 | 7.18 | 7.05 | 1.51 | 10.16 |
| | JG28 | 7.18 | 4.04 | 5.75 | 5.72 | -2.38 | -0.82 |
| | JH20 | 8.35 | 11.19 | 7.58 | 7.25 | -1.72 | 5.82 |
| | JL43 | 1.62 | 5.54 | 7.20 | 9.45 | -2.26 | 0.22 |
| | KD44 | 4.58 | 3.21 | 0.37 | 4.49 | -0.82 | 1.85 |
| | KK22 | 7.99 | 3.32 | 3.63 | 2.44 | 4.96 | 1.78 |
| | LD34 | 8.87 | 5.97 | 11.02 | 9.25 | -0.87 | -3.41 |
| | MA19 | 0.52 | -1.43 | 6.22 | 13.55 | 5.11 | -0.71 |
| | PP21 | 3.02 | 8.80 | 1.90 | 3.25 | 5.64 | 1.39 |
| | SJ52 | 3.19 | 6.43 | 5.73 | 7.02 | 1.28 | 1.53 |
| | SK38 | 6.08 | 1.49 | 4.35 | 3.91 | 1.53 | 6.40 |
| | SL30 | 12.92 | 6.85 | 4.55 | 6.13 | 1.31 | -1.19 |
| | SL46 | 1.92 | 1.23 | 0.20 | 9.36 | 3.89 | 13.67 |
| | WF36 | 1.30 | 6.65 | 1.32 | 7.32 | -5.60 | -1.93 |
| | ZK23 | 7.89 | 8.27 | 11.75 | 12.28 | 2.13 | -6.60 |
| Subject | A1 48HR | L1 1HR | L1 24HR | L1 48HR | | | |
| | FMDΔ | FMDΔ | FMDΔ | FMDΔ | | | |
| | AC22 | -3.96 | -2.25 | -1.83 | -0.12 | | |
| | CB59 | 1.20 | -4.06 | 0.00 | -1.54 | | |
| | DB21 | -1.92 | 0.79 | 0.23 | -3.90 | | |
| | DC26 | -3.52 | -0.19 | 2.88 | -1.15 | | |
| | DH24 | 4.94 | 0.24 | 0.32 | -1.56 | | |
| | JG28 | -5.88 | 2.01 | -1.13 | 0.55 | | |
| | JH20 | -3.38 | -3.14 | -0.30 | -4.24 | | |

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|------|-------|-------|-------|-------|
| JL43 | 0.29 | -4.09 | -0.17 | 3.75 |
| KD44 | 2.34 | 3.21 | 1.84 | 3.12 |
| KK22 | 0.66 | 2.84 | -1.83 | -2.70 |
| LD34 | 0.35 | 3.99 | 1.09 | 4.38 |
| MA19 | 2.86 | -4.67 | -6.63 | 8.36 |
| PP21 | -2.23 | 2.33 | 8.10 | 2.55 |
| SJ52 | 12.76 | -6.62 | -3.38 | -2.79 |
| SK38 | 0.10 | 0.95 | -3.64 | -1.22 |
| SL30 | -1.70 | 4.33 | -1.74 | -2.45 |
| SL46 | 7.75 | -6.70 | -7.39 | 0.73 |
| WF36 | -1.21 | -6.79 | -1.44 | -0.77 |
| ZK23 | 0.09 | -4.78 | -4.40 | -0.39 |

| Subject | A1Pre FlowΔ | A1Post FlowΔ | A2Pre FlowΔ | A2Post FlowΔ | AF FlowΔ | L1Pre FlowΔ |
|---------|----------------|-----------------|----------------|-----------------|-------------|----------------|
| AC22 | 45.1 | 41.4 | 64.2 | 68.0 | 49.2 | 53.0 |
| CB59 | 33.5 | 56.2 | 52.9 | 67.2 | 78.7 | 72.2 |
| DB21 | 22.6 | 34.3 | 61.8 | 37.9 | 24.3 | 29.2 |
| DC26 | 63.5 | 65.5 | 60.3 | 63.6 | 74.4 | 62.2 |
| DH24 | 9.5 | 35.2 | 24.6 | 23.4 | 29.3 | 55.1 |
| JH20 | 42.7 | 46.8 | 82.3 | 58.7 | 49.4 | 33.4 |
| KD44 | 48.3 | 57.2 | 44.7 | 64.5 | 37.5 | 27.3 |
| KK22 | 32.2 | 55.3 | 36.4 | 56.1 | 72.5 | 51.2 |
| LD34 | 41.8 | 63.7 | 48.8 | 60.9 | 44.4 | 59.1 |
| MA19 | 85.5 | 29.9 | 69.1 | 64.7 | 64.5 | 35.4 |
| PP21 | 75.1 | 66.2 | 65.3 | 63.4 | 70.1 | 59.0 |
| SJ52 | 48.2 | 60.0 | 49.6 | 51.2 | 40.0 | 53.5 |
| SK38 | 47.5 | 53.5 | 56.0 | 60.0 | 31.3 | 39.0 |
| SL30 | 55.7 | 54.0 | 60.9 | 62.7 | 61.6 | 51.9 |
| ZK23 | 41.7 | 41.9 | 31.3 | 25.1 | 64.1 | 63.9 |

| Subject | L1Post FlowΔ | L2Pre FlowΔ | L2Post FlowΔ | LF FlowΔ |
|---------|-----------------|----------------|-----------------|-------------|
| AC22 | 48.2 | 54.6 | 56.6 | 50.7 |
| CB59 | 56.9 | 50.4 | 39.8 | 48.0 |
| DB21 | 20.2 | 42.9 | 36.7 | 42.7 |
| DC26 | 58.6 | 59.6 | 47.0 | 62.5 |
| DH24 | 44.2 | 25.2 | 36.2 | 39.1 |
| JH20 | 46.9 | 36.2 | 50.0 | 49.2 |
| KD44 | 64.9 | 59.3 | 59.9 | 45.0 |
| KK22 | 55.9 | 53.0 | 47.4 | 37.3 |
| LD34 | 45.7 | 52.7 | 52.8 | 64.4 |

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|------|------|------|------|------|
| MA19 | 36.1 | 68.6 | 57.9 | 53.8 |
| PP21 | 57.9 | 40.3 | 8.3 | 49.5 |
| SJ52 | 41.8 | 52.9 | 61.1 | 70.5 |
| SK38 | 49.5 | 54.7 | 39.9 | 47.4 |
| SL30 | 70.6 | 45.8 | 61.1 | 42.0 |
| ZK23 | 53.2 | 52.4 | 74.3 | 34.5 |

| Subject | A1Pre PWV | A1Post PWV | A2Pre PWV | A2Post PWV | AF PWV | L1Pre PWV |
|---------|--------------|---------------|--------------|---------------|-----------|--------------|
| AC22 | 7.95 | 10.19 | 8.09 | 8.68 | 7.82 | 6.51 |
| CB59 | 10.42 | 6.90 | 9.77 | 8.85 | 7.82 | 8.68 |
| DB21 | 5.65 | 5.65 | 6.60 | 5.79 | 5.58 | 6.34 |
| DC26 | 6.17 | 5.62 | 5.15 | 6.09 | 5.39 | 5.27 |
| JG28 | 6.80 | 8.23 | 7.82 | 7.11 | 8.53 | 7.44 |
| JH20 | 5.27 | 5.94 | 6.25 | 6.60 | 6.42 | 5.45 |
| JL43 | 5.87 | 7.95 | 7.00 | 6.60 | 6.42 | 7.82 |
| KD44 | 6.17 | 6.51 | 5.94 | 5.86 | 7.11 | 6.09 |
| LD34 | 5.86 | 6.34 | 5.72 | 5.21 | 5.86 | 5.93 |
| MA19 | 5.21 | 5.65 | 6.51 | 5.79 | 5.28 | 5.33 |
| PP21 | 5.56 | 5.94 | 5.15 | 5.27 | 5.45 | 5.94 |
| SJ52 | 6.34 | 5.52 | 6.25 | 5.58 | 6.01 | 6.09 |
| SK38 | 7.21 | 8.37 | 6.70 | 7.82 | 6.17 | 6.01 |
| SL30 | 5.94 | 5.58 | 5.38 | 5.39 | 5.10 | 6.80 |
| SL46 | 9.03 | 8.53 | 8.53 | 7.33 | 7.56 | 8.68 |
| ZK23 | 6.09 | 6.60 | 6.29 | 5.01 | 5.79 | 6.34 |

| Subject | L1Post PWV | L2Pre PWV | L2Post PWV | LF PWV | A1 1HR PWVΔ | A1 24HR PWVΔ |
|---------|---------------|--------------|---------------|-----------|----------------|-----------------|
| AC22 | 5.94 | 6.60 | 6.90 | 9.02 | 2.2 | 0.1 |
| CB59 | 7.95 | 8.23 | 8.53 | 8.23 | -3.5 | -0.7 |
| DB21 | 6.64 | 5.72 | 6.51 | 6.51 | 0.0 | 1.0 |
| DC26 | 5.33 | 4.99 | 5.72 | 5.04 | -0.6 | -1.0 |
| JG28 | 7.21 | 7.11 | 7.69 | 7.21 | 1.4 | 1.0 |
| JH20 | 6.09 | 6.34 | 5.39 | 6.38 | 0.7 | 1.0 |
| JL43 | 5.45 | 5.93 | 6.17 | 7.95 | 2.1 | 1.1 |
| KD44 | 5.72 | 7.08 | 6.34 | 6.70 | 0.3 | -0.2 |
| LD34 | 5.26 | 5.29 | 5.72 | 5.42 | 0.5 | -0.1 |
| MA19 | 5.65 | 6.70 | 5.15 | 5.45 | 0.4 | 1.3 |
| PP21 | 5.58 | 5.27 | 5.27 | 5.27 | 0.4 | -0.4 |
| SJ52 | 5.79 | 6.09 | 5.94 | 6.01 | -0.8 | -0.1 |
| SK38 | 6.90 | 7.33 | 6.70 | 7.21 | 1.2 | -0.5 |
| SL30 | 5.33 | 5.45 | 5.33 | 5.39 | -0.4 | -0.6 |

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|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| SL46 | 7.33 | 6.80 | 7.44 | 7.69 | -0.5 | -0.5 |
| ZK23 | 6.70 | 4.94 | 5.58 | 5.65 | 0.5 | 0.2 |
| Subject | A1 48HR PWVΔ | L1 1HR PWVΔ | L1 24HR PWVΔ | L1 48HR PWVΔ | | |
| AC22 | -0.1 | -0.6 | 0.1 | 2.51 | | |
| CB59 | -2.6 | -0.7 | -0.5 | -0.46 | | |
| DB21 | -0.1 | 0.3 | -0.6 | 0.18 | | |
| DC26 | -0.8 | 0.1 | -0.3 | -0.23 | | |
| JG28 | 1.7 | -0.2 | -0.3 | -0.23 | | |
| JH20 | 1.2 | 0.6 | 0.9 | 0.93 | | |
| JL43 | 0.6 | -2.4 | -1.9 | 0.13 | | |
| KD44 | 0.9 | -0.4 | 1.0 | 0.61 | | |
| LD34 | 0.0 | -0.7 | -0.6 | -0.51 | | |
| MA19 | 0.1 | 0.3 | 1.4 | 0.12 | | |
| PP21 | -0.1 | -0.4 | -0.7 | -0.67 | | |
| SJ52 | -0.3 | -0.3 | 0.0 | -0.08 | | |
| SK38 | -1.0 | 0.9 | 1.3 | 1.20 | | |
| SL30 | -0.8 | -1.5 | -1.3 | -1.41 | | |
| SL46 | -1.5 | -1.4 | -1.9 | -1.00 | | |
| ZK23 | -0.3 | 0.4 | -1.4 | -0.69 | | |
| Subject | A1 IPE PVA | A1 1HR PVA | A2 PRE PVA | A2 IPE PVA | A2 1HR PVA | AF PVA |
| DB21 | -9.012 | -7.099 | -3.899 | -7.622 | -10.557 | -12.031 |
| DC26 | -2.594 | -1.679 | 3.383 | 4.787 | -0.549 | 1.903 |
| DH24 | -5.465 | 3.733 | 5.651 | -4.796 | 3.029 | 5.659 |
| JG28 | 2.094 | -3.787 | 0.142 | 1.112 | 0.741 | 4.848 |
| JL43 | 5.341 | 2.407 | 3.291 | 0.769 | -0.828 | -11.827 |
| KD44 | -4.406 | -0.895 | 4.532 | -0.171 | -2.439 | 5.388 |
| LD34 | -2.349 | -2.254 | -4.919 | -8.200 | -6.869 | -4.349 |
| MA19 | -6.391 | -8.575 | 0.839 | -1.005 | 2.391 | -3.632 |
| PP21 | -3.015 | -7.402 | -4.447 | -6.804 | -12.495 | -5.860 |
| SJ52 | 3.214 | 6.730 | 13.027 | 4.471 | 6.915 | 6.915 |
| SL46 | -6.151 | -6.915 | 1.247 | -5.979 | -10.014 | 2.416 |
| WF36 | -4.541 | 0.777 | 9.943 | 8.304 | 1.863 | 12.905 |
| ZK23 | -5.512 | -4.022 | 7.766 | -0.139 | -0.476 | 7.787 |
| Subject | A1 Pre Adj NO | A1 IPE Adj NO | A1 1hr Adj NO | A2 Pre Adj NO | A2 IPE Adj NO | A2 1hr Adj NO |
| DB21 | 4.678 | 4.174 | 4.244 | 3.381 | 3.585 | 2.612 |
| DC26 | 4.261 | 3.250 | 2.319 | 2.776 | 3.914 | 3.301 |

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|----------------|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| DH24 | 4.580 | 4.728 | 4.730 | 5.093 | 4.666 | 5.525 |
| JG28 | 2.552 | 2.502 | 2.011 | 2.052 | 1.849 | 2.469 |
| JL43 | 2.992 | 2.891 | 2.495 | 6.151 | 7.348 | 6.660 |
| KD44 | 12.765 | 11.475 | 10.551 | 6.268 | 4.959 | 4.886 |
| LD34 | 4.123 | 4.730 | 4.493 | 2.258 | 3.068 | 3.112 |
| MA19 | 1.483 | 1.820 | 1.997 | 1.421 | 1.724 | 1.727 |
| PP21 | 3.731 | 3.407 | 3.279 | 1.846 | 2.083 | 2.055 |
| SJ52 | 4.607 | 4.828 | 4.595 | 2.842 | 2.072 | 2.556 |
| SL46 | 1.912 | 1.395 | 1.433 | 1.756 | 1.781 | 2.247 |
| WF36 | 5.985 | 4.918 | 6.146 | 9.599 | 8.082 | 7.640 |
| ZK23 | 10.624 | 8.531 | 8.767 | 4.983 | 4.448 | 2.798 |
| Subject | AF Adj NO | L1 IPE PVA | L1 1HR PVA | L2 PRE PVA | L2 IPE PVA | L2 1HR PVA |
| DB21 | 1.629 | -4.372 | -4.731 | -0.148 | -3.118 | -3.663 |
| DC26 | 4.194 | -1.676 | -3.332 | 4.048 | 2.316 | 1.151 |
| DH24 | 6.982 | -2.647 | 0.341 | 12.101 | 3.583 | -4.228 |
| JG28 | 2.317 | -8.197 | -0.581 | 6.031 | -1.181 | -0.765 |
| JL43 | 3.491 | 1.601 | -2.941 | 4.189 | 5.569 | 8.602 |
| KD44 | 16.229 | -8.063 | -4.918 | 6.226 | -0.671 | 4.796 |
| LD34 | 4.141 | -0.862 | -3.990 | -0.748 | 3.093 | 3.265 |
| MA19 | 1.092 | -2.799 | -3.818 | -5.691 | -8.348 | -2.156 |
| PP21 | 1.712 | 2.668 | -1.801 | 3.558 | 3.539 | -1.801 |
| SJ52 | 3.030 | 1.677 | 5.792 | 11.721 | 4.477 | 4.583 |
| SL46 | 5.644 | -9.430 | -4.977 | -9.464 | -14.532 | -7.853 |
| WF36 | 4.982 | -4.517 | 3.718 | -2.738 | -2.794 | -8.903 |
| ZK23 | 8.195 | -3.220 | -0.407 | 15.139 | 13.214 | 9.884 |
| Subject | LF PVA | L1 Pre Adj NO | L1 IPE Adj NO | L1 1hr Adj NO | L2 Pre Adj NO | L2 IPE Adj NO |
| DB21 | -7.240 | 2.250 | 2.602 | 2.161 | 1.668 | 1.899 |
| DC26 | -4.646 | 3.174 | 2.871 | 3.523 | 3.152 | 3.062 |
| DH24 | 4.800 | 8.580 | 9.625 | 8.134 | 10.191 | 8.887 |
| JG28 | 3.099 | 3.656 | 3.633 | 3.655 | 3.493 | 2.720 |
| JL43 | 2.817 | 7.827 | 6.928 | 6.119 | 5.883 | 5.787 |
| KD44 | 10.149 | 4.028 | 3.782 | 3.911 | 3.771 | 5.227 |
| LD34 | 8.045 | 3.671 | 3.721 | 3.248 | 1.601 | 2.851 |
| MA19 | -1.503 | 1.723 | 1.962 | 2.012 | 1.660 | 3.152 |
| PP21 | 9.821 | 5.398 | 5.425 | 4.966 | 3.315 | 2.588 |
| SJ52 | -4.657 | 4.767 | 5.063 | 4.330 | 4.196 | 3.961 |
| SL46 | -4.564 | 7.973 | 6.714 | 6.087 | 3.198 | 3.035 |
| WF36 | -9.661 | 2.992 | 3.436 | 3.398 | 4.237 | 3.921 |

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|----------------|--------------------------|----------------------|-------|-------|-------|-------|
| ZK23 | 19.920 | 4.986 | 6.081 | 4.775 | 7.676 | 6.994 |
| Subject | L2 1hr Adj NO | LF Adj NO | | | | |
| DB21 | 1.609 | 2.205 | | | | |
| DC26 | 2.386 | 2.491 | | | | |
| DH24 | 8.852 | 3.831 | | | | |
| JG28 | 2.871 | 1.865 | | | | |
| JL43 | 7.026 | 4.980 | | | | |
| KD44 | 5.805 | 7.695 | | | | |
| LD34 | 3.876 | 3.344 | | | | |
| MA19 | 1.812 | 1.479 | | | | |
| PP21 | 2.641 | 6.822 | | | | |
| SJ52 | 3.872 | 3.733 | | | | |
| SL46 | 2.877 | 2.107 | | | | |
| WF36 | 3.468 | 7.306 | | | | |
| ZK23 | 6.905 | 11.031 | | | | |