THESIS

CARDIOVASCULAR DISEASE RISK IN MIDDLE-AGED ULTRA-ENDURANCE ATHLETES

Submitted by

Nate P. Bachman

Department of Health and Exercise Science

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Master's Committee:

Advisor: Frank Dinenno
Co-advisor: Jennifer Richards

Adam Chicco
Christopher Orton

ABSTRACT

CARDIOVASCULAR DISEASE RISK IN MIDDLE-AGED ULTRA-ENDURANCE ATHLETES

Background: It is widely accepted that aerobic exercise has the ability to reduce cardiovascular disease (CVD) risk. However, recent studies suggest that volumes of exercise that greatly exceed physical activity guidelines may be damaging to the heart. Currently, it is unclear if individuals who train for ultra-endurance races are at an elevated risk of developing CVD compared to those that perform lower amounts of physical activity. *Purpose:* To use traditional and novel measures of CVD risk to determine if individuals that train for ultra-endurance races have a greater CVD risk compared to participants that engage in recreational physical activity. *Methods:* We studied two groups of healthy, middle-aged adults (40-65 y); Control (CON, n=18) subjects included individuals who were meeting current physical activity guidelines and the athletes (ATH, n=25) had been training for ultra-endurance events for 10 years. We used cardiac computed tomography (CT) to calculate coronary artery calcium scores (CACS) and magnetic resonance imaging (MRI) to assess for myocardial fibrosis (MF). Vascular function was evaluated using carotid-femoral pulse wave velocity (cfPWA) and flow-mediated dilation (FMD). 10-Year coronary heart disease (CHD) risk was also determined using a risk score calculator. *Results:* CACS > 0 was observed in 2 CON and 8 ATH; however, the presence of CAC was not significantly different between groups (P>0.05). Additionally, no participants in CON or ATH had MF. CON had higher cfPWV compared to ATH (6.9±0.2 vs 6.2±0.2 m/s, P<0.05), while no differences in FMD were observed (CON; 5.6±1.2 vs ATH; 3.6±0.8 %,

P>0.05). Furthermore, there were no group differences in CHD risk (CON; 1.6±0.3 vs ATH; 2.4±0.6 %, P>0.05). *Conclusion:* ATH training for ultra-endurance races are not at a greater risk of experiencing a cardiac event than individuals that meeting current physical activity guidelines.

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1. LITERATURE REVIEW

1.1 Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States (70). CVD encompasses a variety of diseases that affect the heart and vasculature, including coronary artery disease (CAD), rhythmic disorders, and heart failure, all of which can develop over the lifespan. While there are numerous modifiable risk factors for developing CVD, aging is the primary risk factor (50). One explanation for aging influencing the development and progression of CVD is the structural and functional changes that take place within the cardiovascular system with age. An approach that appears to be successful in attenuating some of the age-related changes associated with cardiovascular pathology is participation in regular aerobic exercise (89). However, recent reports in humans (1, 30, 58) and rodent models (8, 91) suggest chronic participation in higher volumes (> 10 years of marathon or triathlon training) of aerobic exercise may contribute to cardiac pathogenesis despite the known cardio-protective effect of regular aerobic exercise to offset detrimental age-related cardiovascular risk.

Regular physical activity (PA) is known to promote cardiovascular health by reducing risk factors that contribute to CVD (71). PA guidelines recommend at least 150 minutes of moderate exercise or 75 minutes of vigorous exercise each week (98). PA can also be quantified by using metabolic equivalents (METs), where a MET value is assigned to each activity based on the energy demands of the activity (2). Individuals who engage in approximately 10 MET-h/wk. (MET values from each activity x duration = MET-h/wk.) of physical activity week meet the aforementioned physical activity guidelines (28). By contrast, highly-active exercisers may complete more than 75 MET-h of aerobic training per week (5). Surprisingly, recent large-scale

epidemiological studies have reported that individuals who performed the uppermost volumes of exercise or higher intensity exercise have an elevated mortality risk compared to participants with low or moderate activity levels (93, 121). Moreover, studies examining cardiac function immediately following long endurance events (three to eleven hours in duration) (30) and those examining coronary artery health (coronary artery calcium) and cardiac scarring (fibrosis) in healthy long-term marathon runners and cyclists (1, 58, 63, 84, 85) indicate high volumes of aerobic exercise can impair function acutely and may lead to pathologic morphology overtime in older exercisers. To date, numerous studies have investigated the role of aerobic exercise on vascular function (33), but little is known about the vascular health of the middle-aged and older athletes that demonstrate this potential cardiac maladaptation to training.

The purpose of this review is to briefly examine and summarize the current literature on aging and chronic exercise training on vascular structure and function in middle-aged and older adults as well as the interaction between exercise and the vascular system in these individuals. Additionally, the paradoxical incidence of cardiovascular pathology in highly-trained, older endurance athletes will be described to demonstrate the equivocal relationship between uppermost volumes of aerobic training and CV risk.

1.2 Arterial Structure and Function: the Influence of Aging

Modifications in vascular architecture are central to vascular aging. One such change is the increase in wall thickness of in the common carotid artery (CCA), which is often quantified via ultrasound by measuring the carotid intima-media thickness (CIMT) (44). This morphology appears to be due in part to expansion of the extracellular matrix resulting from excess collagen deposition and smooth muscle hypertrophy in the medial layer of the arterial wall (119). Simultaneously, elastin tends to break down or fragment and collagen may become

cross-linked. The combined changes in collagen and elastin distribution in the vessel wall alter the ratio of these proteins, ultimately contributing to diminished arterial compliance. Importantly, arterial compliance in the abdominal aorta can be estimated non-invasively using a technique known as carotid-femoral pulse-wave velocity (cfPWV). By simultaneously measuring the pulse at the common carotid and femoral arteries, the speed at which the pulse wave travels down the aorta can be approximated, with a greater velocity being indicative of less compliant vessel. Elevations in cfPWV are apparent in aged individuals independent of disease (114).

The changes in vascular structure mentioned above are often thought of as predominant factors that contribute to a decline in arterial compliance. Additionally, alterations in vascular tone may also contribute to the diminished in vascular compliance that accompanies aging. During the aging process, concentrations of circulating vasoconstrictors increase and vasodilators decrease such that a greater basal level of vascular smooth muscle (VSM) contraction is maintained, which may contribute to producing a less compliant phenotype (40, 113). Supporting this notion, elevations in the potent vasoconstrictor endothelin-1 (ET-1) have been observed in aged humans (24). At the same time, bioavailability of the anti-proliferative, anti-atherogenic, vasodilator nitric oxide (NO) falls. One method of evaluating NO in humans is flow-mediated dilation (FMD). This test utilizes Doppler ultrasound to observe a change in vessel diameter in large conduit arteries due to a hyperemic response initiated by dilation of downstream resistance vessels following a brief period of local limb ischemia (5 minutes) (109). The resultant hyperemia following ischemia produces shear stress on the endothelium of the vessel of interest (typically the brachial artery), which stimulates the production of nitric oxide and other dilator molecules. While this test does not directly measure NO production,

pharmacological studies inhibiting NO generation (via LNMMA/L-NAME) have revealed that the dilation of the conduit vessel is predominantly reliant on NO (34). Additionally, studies utilizing antioxidants have demonstrated an acute reversal of endothelial dysfunction (impaired FMD) in older volunteers that is concomitant with lower levels of oxidative stress (123). These data suggest that diminished FMD in these aged individuals is partially due to reactive oxygen species scavenging of NO. Further, experiments that investigate differences in vascular function often incorporate the use of "endothelium-independent" dilators, substances that are administered intra-arterially and act directly on the VSM, demonstrate preserved smooth muscle function in the presence of impaired FMD (10, 53, 72). These studies reveal that the FMD technique specifically targets endothelial mediated vascular function/dysfunction. Cross-sectional studies have demonstrated that FMD values, expressed as a percent change in vessel diameter from baseline, are often lower in older compared to young adults (10, 11, 110). Taken together, inability to generate NO and/or the lack of bioavailability of NO within the vascular endothelium play a role in the decline in FMD that accompanies age.

1.3 Arterial Structure and Function: The Impact of Exercise and Disease

Habitual aerobic exercise appears to have a positive influence on some aspects of vascular structure and function. Indeed, structural differences are often observed in the vasculature of athletic populations when compared to their sedentary peers. Both cross-sectional and interventional studies indicate endurance training produces larger conduit artery diameters and lower wall thickness values in vessels such as the brachial and femoral, and these adaptations appear to be based on activity type (i.e., activities involving upper or lower limbs) (22, 118). Importantly, these changes are not known to offset disease development or

progression. Alterations in conduit artery architecture are likely an effort to enhance blood supply to active muscle and normalize shear stress on the vascular endothelium (36).

Surprisingly, similar decreases in wall thickness are not present in the common carotid artery (i.e., CIMT) in endurance trained middle-aged and older men and women compared to their sedentary peers (67, 104, 112). Nonetheless, it is still important to delineate between normal CIMT measures and excessive wall thickening that accompany disease. CIMT values that are above normative values for age and sex are associated increased CVD event incidence and CCA plaque development (13, 44, 76, 92). Taken together, elevations in CIMT in aged individuals represents structural changes in factors such as the increased collagen and VSM proliferation within the arterial wall. Conversely, abnormal values may be reflective of subclinical atherosclerosis or other advanced cardiovascular pathogenesis.

Unlike CIMT, CCA compliance, the ability of the vessel to distend to a pulse of blood and subsequently recoil, is influenced by physical activity and is enhanced in middle-aged and older habitually trained men and women compared to sedentary controls (65, 66, 103). The lack of difference in wall thickness despite disparate measures of compliance between aerobically-trained and sedentary older adults indicate the changes in the CCA wall that influence vascular function may not be not homogenous across individuals with different activity levels (i.e., more smooth muscle in athletes vs more collagen in sedentary individuals). However, this potential divergence in carotid wall composition cannot be detected non-invasively (ultrasonography). Alternately, differences in CCA between regular exercisers and sedentary adults may be due to differences in resting vascular tone (99, 100).

Other assessments of vascular function of aging athletes in cross-sectional studies appear to parallel preservation of CCA compliance. Measurements of cfPWV in older endurance-

trained adults approaching the seventh decade are similar to values observed in young, healthy individuals nearly 40 years younger (114). Additionally, it appears that maintaining an active lifestyle can offset the age-related rise in cfPWV in both men and women (29, 97, 102). This relationship between aerobic fitness and cfPWV demonstrates how regular exercise can preserve vascular elasticity, thus diminishing the aortic pulse wave. However, there is currently sparse evidence that cfPWV can be lowered using exercise interventions in older adults (78). This indicates that aerobic exercise may be able to prevent but not reverse age-related aortic stiffening. Nonetheless, much higher than average cfPWV values are often found in adults with cardiovascular risk factors such as hypertension, dyslipidemia, and diabetes (51). Furthermore, multiple population-based studies have demonstrated that elevated cfPWV values are associated with an increased risk of cardiovascular mortality, coronary heart disease, and stroke (7, 60, 101). Due in part to the heterogeneous nature of the elastic properties of the arterial tree, it is unclear whether increased vascular stiffness represented by faster cfPWV values precede or are the result of the progression of CVD. Regardless, these measurements of aortic stiffness appear to be related to the pathogenesis of CVD and are related to CVD risk, which may be offset by long-term participation in regular aerobic exercise.

FMD is another measure of vascular health that can be improved with aerobic training. Due to its capacity for improvement, this assessment is often used as an end-point measure of exercise intervention studies that seek to rescue diminished vascular function in older participants. Cross-sectional data reveal that chronically endurance trained older participants often have superior FMD values compared to their sedentary peers, while exercise training of sedentary older participants results in enhanced FMD (10, 11, 80). It is important to note that the improvements in endothelial function determined by FMD may be sex specific in older adults,

with little to no benefit observed amongst trained post-menopausal women (69, 80, 90). Nonetheless, lower FMD values that are thought to reflect dysfunctional or diseased vascular endothelium are also observed in patient populations including peripheral artery disease and coronary artery disease (CAD) (53, 72). Moreover, low FMD values can be predictive of CVD events in patient and non-patient populations (15, 95, 124, 125). The prevalence of low FMD in individuals with chronic disease and the elevated risk of experiencing a CVD event indicates that changes to brachial artery function may reflect pathogenesis within the coronary vasculature or other locations along the vascular tree. To support this notion, impaired FMD values have been observed in CAD patients admitted to the hospital for catheterization (4). In these patients, reduced vasodilatory responses to pharmacological stimulation of the coronary arteries have been associated with diminished FMD values. The growing body of evidence supports that FMD is positively related to exercise training and is a useful tool in evaluating underlying CV event risk (37).

1.4 "Extreme" Levels of Endurance Training: An Emerging CV Risk Factor?

The ability of aerobic exercise to mitigate or reverse age and disease-related changes in vascular function reveals how exercise may enhance health beyond the benefit associated with modifying traditional CV risk factors (33). Furthermore, the improvements in vascular function that result from or are concomitant with exercise training help explain why changes in some measures of vascular function are related to CV event risk reduction (37). While experimental studies typically use moderate amounts of exercise (30-60 min/day, 3-6 days/wk) to examine the direct impact of exercise on vascular function (10, 103, 104, 112), some ultra-endurance athletes, individuals training for competitions greater than six hours in duration, perform seven to twelve hours of aerobic training each week (48). In the context of PA recommendations for health,

these individuals may be performing five to ten times more exercise than what is deemed necessary to prevent disease (5). Generally, a dose-dependent response for exercise and mortality risk is observed where higher levels of volume are associated with the greatest reduction in risk (105). However, recent data suggests that there may be lack of risk reduction or increased risk associated with the highest volumes of aerobic training such as those that accompany ultra-endurance training (5, 30, 58). Additionally, epidemiological studies that have stratified large groups of participants by activity level have observed an elevation in mortality risk associated with higher exercise volumes and intensities when compared to more moderate levels of activity. For instance, Williams et al. (2014) found that self-identified heart attack survivors who engaged in > 50 MET-h/wk of running or walking had a more than two and a half times higher mortality risk compared to those survivors that completed a lower amount of aerobic exercise (38-50 MET-h/wk.). In a different study, Schnohr et al. found that risk of mortality was comparable for individuals in the strenuous running group (running speed of ~ seven mph) and sedentary non-running, while risk was lower for light and moderate intensity runners (running of speed of ~ five mph) compared to sedentary subjects or those who jogged strenuously (93). While existing pathology within the participants in Williams et al.'s (2014) study may have contributed to mortality risk, taken together, these studies suggest that the combination high-volume and high-intensity aerobic activity may diminish the cardio-protective effect that accompanies endurance exercise.

Numerous questions remain about the nature of the relationship between the highest volumes of aerobic training and cardiovascular risk. Multiple studies have demonstrated that high-intensity aerobic exercise can lead to an acute and transient heightened risk of experiencing a cardiac event (3, 61). These data reveal that vigorous effort activities temporarily raise risk

during and immediately following an exercise bout. However, this short-term risk appears to also be associated with baseline fitness levels, and habitual exercisers still experience an overall reduction in risk from exercise participation compared to less fit individuals. It has been suggested that this brief period of amplified risk may be related to plaque rupture leading to myocardial ischemia or results from the rise in sympathetic activity that accompanies exercise triggering fatal arrhythmias. Alternatively, recent investigations have begun to examine whether long-term training leads to irreversible structural changes in the coronary vasculature and myocardium that may contribute to an elevated risk of a future CV events (1, 58, 63, 84, 85). One area of interest is the incidence of coronary artery calcium (CAC) in otherwise healthy, asymptomatic exercisers. Typically, CAC accompanies atherosclerosis and intimal CAC results from the calcification of coronary artery plaque, with the total amount of CAC correlating with overall plaque volume (56). These pathological changes contribute to arterial hardening and stenosis, which elevates the risk of experiencing a cardiac event. Therefore, it is expected that CAC should be low in habitually-trained aerobic athletes due to the positive effect that endurance training has on risk factors for coronary plaque development. Surprisingly, greater amounts of CAC have been observed in middle-aged aerobically-trained males and have been associated with increasing exercise volume and intensity (1), which would normally be expected to contribute to greater risk reduction (105). However, some older athletes that have calcium deposition also have more risk factors for coronary disease (84) and appear to have a similar levels of calcification as controls when matched for risk factors (63). Additionally, only few studies (58, 85) have included endurance-trained females, who appear to a similar amounts of CAC compared active or sedentary controls. Currently, it is unclear if the exercise participation

is independently contributing to CAC development exclusively in men or if the presence of CAC in older athletes can be attributed to CAD risk factors.

Another marker indicative of cardiac pathology that has been observed in endurance athletes is myocardial fibrosis (MF). MF occurs when the myocardium is damaged as the result of ischemia or infection (23). In order to maintain cardiac structure, the damaged cardiomyocytes are replaced by collagen. However, ischemia and infection are not the only stimuli that can result in MF. When the heart is presented with a chronic pressure overload, such as the increase in afterload that the right ventricle faces during pulmonary hypertension, reactive interstitial fibrosis can lead to deposition of collagen within the atrial or ventricular extracellular matrix (87). Along these lines, some investigators believe that long-duration, high-intensity aerobic training may produce similar pressure challenges to the hearts of highly-trained endurance athletes, leading to fibrosis. Fibrosis can be detected by cardiac magnetic resonance imaging (MRI) and is clinically valuable because it is predictive of ventricular arrhythmias. This prognostic value has generated interest in using cardiac MRI for studies investigating potential maladaptation to endurance training. Mohlenkamp and colleagues (2008) examined the incidence of MF in middle-aged male marathon runners and found 12% of these athletes to have fibrosis. Similarly, Merghani et al. (2017) found 14% of male participants (runners and cyclists) to have MF in a study of middle-aged male and female athletes. Importantly, none of the control subjects in this study displayed evidence of MF. Although these studies found similar percentages of MF, only Mohlenkamp et al. found fibrosis to be correlated with number of marathons run (63) while the study from Merghani et al. did not find a relationship between MF and exercise intensity, years of training, or number of competitions completed (58). Nonetheless, preclinical models of maladaptation to endurance training have demonstrated a

graded response of fibrosis between sedentary, moderate exercise (45 min. at ~60 % VO_2 max , 5 days/wk), and high-load exercise (1 hr. at ~85 % VO2 max 5 days/wk) (91) resulting from long-term running. Differences in fibrotic patterns among athletes in human studies suggests that at least some MF may be explained by previous cardiac injury (i.e., ischemia, infection) rather than the consequence of training and competition (58, 63). However, the preclinical data combined with the inability of prior myocardial damage to explain all MF observed in older athletes are compelling evidence suggesting that some MF may be the consequence of endurance training.

1.5 Statement of the Problem

A large body of evidence demonstrates the positive benefits of regular aerobic exercise. However, recent evidence suggests that volumes of endurance training that greatly exceed physical activity recommendations for general health may lead to adverse cardiac morphology overtime. Currently, it is unclear if these changes in cardiac structure affect the risk of having a future cardiac event in chronically-trained middle-aged adults, and if men and women are affected similarly. Additionally, little is known how other divisions of the cardiovascular system such as the central elastic arteries (aorta, carotid) and peripheral conduit vessels (brachial) are impacted in individuals that demonstrate maladaptive responses to habitual endurance training.

1.6 Hypothesis

The purpose of this study was to test the hypothesis that middle-aged older adults training for ultra-endurance events have more CAC than controls and this difference would be exclusive to men. Furthermore, we hypothesized that athletes would exhibit a greater amount of fibrosis which would also accompany impaired measures of vascular function compared to controls.

2. INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death and disability in the United States. Importantly, regular physical activity (PA) is an effective means to modify CVD risk factors (e.g., circulating lipids, blood pressure, obesity, etc.), thus reducing CVD risk (32, 47). Furthermore, aerobic exercise appears to confer health benefits beyond those of reducing risk factors alone, leading to the notion of the "risk factor gap" (6, 46, 73). For instance, alteration of risk factors by exercise only accounts for up to 60% of risk of CVD (46), indicating that other causes beyond these established variables contribute to CVD pathogenesis.

Vascular adaptations to exercise training may help account for the benefit of PA not explained by risk factor reduction. Specifically, measures of central and peripheral vascular function such as carotid-femoral pulse wave velocity (cfPWV) and flow-mediated dilation (FMD), respectively, are related to disease risk (7, 37, 60, 80, 101, 114, 124, 125) and respond to exercise interventions (9, 25, 80, 82), making them potential candidates to explain the risk factor gap. Benefits from regular physical activity are gained in a dose-dependent fashion (6, 105), and higher volumes of exercise (e.g., endurance training vs recreational physical activity) may be required to observe a greater risk reduction associated with vascular adaptations to exercise. Paradoxically, some recent data suggests that a rise in CVD risk due to pathologic cardiac structure may be associated with increasing levels of physical activity (5, 93, 121, 126).

Observational studies in humans have demonstrated the presence of coronary calcium in endurance-trained middle-aged adults (1, 58, 63, 84, 85). Coronary artery calcium (CAC) correlates with total plaque burden (88) and is associated with elevated risk of development of CVD or experiencing a cardiac event (20, 57, 81). While population-based studies report an

inverse relationship between cardiorespiratory fitness and CAC in younger adults (52), increasing CAC has been correlated with exercise volume and intensity (1) and more years of training (58, 84) in middle-aged adults. However, some athletes have similar amounts of calcification as control participants when matched for cardiac risk factors (63), leaving questions about the nature of the relationship between CAC and exercise. Additionally, conflicting reports of lower (85) or similar (58) amounts of CAC in female masters athletes compared to control participants demonstrates potential sex differences in the pathogenesis of coronary calcification in trained adults.

CAC has also been associated with the presence of myocardial fibrosis in masters athletes (63). MF often occurs in response to ischemic injury or infection, resulting in collagen deposition within the myocardium (23). Clinically, assessment of MF is valuable due to its ability to be predictive of future cardiac events in some patient populations (49, 59, 75). The current literature on MF indicates middle-aged male runners and cyclists (58, 94) may demonstrate greater amounts of fibrosis than controls, but this relationship is has been under studied and less clear in women. Interestingly, rodent models of overtraining support a cause and effect relationship between exercise volume and intensity with alterations in cardiac function, arrhythmogenesis, and MF (8, 91). Nonetheless, divergent patterns of MF in humans indicate that some of these cases may not be exercise related (58, 94).

In summary, the possibility that endurance training in masters athletes exerts a detrimental impact on cardiac health is a dramatic departure from the well-documented positive influence of regular physical activity on vascular function measured by cfPWV (102, 114) and FMD (11, 74, 80, 86), although some of these adaptations may be limited to men when studying middle-aged adults (69, 80, 90). Therefore, the purpose of this study was to assess cardiac structure by

measuring CAC and myocardial fibrosis in middle-aged long-term (≥10 years) ultra-endurance athletes and healthy, active controls to determine the role of exercise in elevated cardiac risk. Using traditional measures of cardiac risk along with imaging data, we were then able to calculate 10-year cardiac event risk. We also evaluated vascular function using brachial artery FMD, cfPWV, and carotid compliance to establish whether maladaptive cardiac morphology coincided with impaired vascular function. Additionally, we wanted to examine potential sex differences in cardiac structure and vascular function related to training status. We hypothesized that athletes would have more CAC than controls and this difference would be exclusive to men. Furthermore, we hypothesized that athletes would exhibit a greater amount of fibrosis which would also accompany impaired measures of vascular function compared to controls.

3. METHODS

3.1 Subjects

Healthy volunteers between the ages of 40-65 were recruited. Participants were grouped into two categories: athletes (ATH) and control (CON). Individuals in the ATH group had a minimum of 10 years of competitive or recreational cycling, running, swimming, or triathlon aerobic training. Most participants competed annually in endurance events such as long-distance cycling races, ultramarathons, and Ironman triathlons. ATH with a prolonged period of inactivity (>3 months per year) were excluded. Subjects included in the CON group performed regular mixed exercise including weights, jogging, walking, and recreational sports. Participants were recruited using flyers sent to local cycling, running, and triathlon groups as well as community centers and local businesses.

All subjects were free of overt cardiovascular, metabolic, and other chronic diseases. Exclusion criteria included current or previous treatment for hypertension (defined as systolic >140mmHg or diastolic >90mmHg), dyslipidemia, diabetes mellitus (type I or II), cancer, or renal disease. Participants with a history of regular cigarette smoking were also excluded.

Exercise Volume. For the ATH, average weekly exercise volume by activity, years of training, and competitions completed were determined using questionnaires. Exercise volume was quantified by assigning metabolic equivalent (MET) values to each reported training activity (1, 2). Physical activity for CON participants was based on the International Physical Activity Questionnaire (IPAQ), a survey validated to use for the assessment of PA across a variety of populations (19). MET values of each activity for each participant were then multiplied by the number of hours spent performing that respective activity (98). Total weekly MET-h was

calculated for each participant (Table 1). Inclusion criteria for PA in the ATH group was ≥ 100 MET-h per week. Control participants were required to complete a volume of weekly exercise equivalent to the minimum of 10 MET-h (150 minutes of moderate exercise), based on current PA guidelines (98).

3.2 General Health Assessment, Exercise Testing, and Body Composition

Participants visited the Human Performance Clinical Research Laboratory at Colorado State University on two occasions. One visit included a graded exercise test (GXT) and the other visit consisted of assessments to evaluate vascular structure and function.

Health History Questionnaire. Screening for chronic disease and cardiac risk factors were determined by use of a health history survey.

Electrocardiogram. Cardiac rhythm and heart rate were monitored at rest and during each stage of exercise testing using a 12-lead electrocardiogram (ECG; Mortara Instrument, Inc.; Milwaukee, WI, USA). The highest observed heart rate during exercise testing was reported as 'Peak Heart Rate.'

Physician Assessment. A physical examination and resting ECG, heart rate, blood pressure were assessed by a licensed cardiologist prior to exercise testing. All exercise ECG tracings were evaluated for cardiac arrhythmias and signs of ischemia by the physician following the aerobic capacity test.

Aerobic Capacity. VO_2 Peak was determined during a GXT using a cycle ergometer (Lode B.V.; Groningen, NL). Participants completed a staged incremental exercise protocol that increased the work-rate by 25 watts every two minutes until volitional fatigue was reached. Power output achieved during the final stage of exercise was reported as 'Peak Watts.' Breath-by-breath oxygen consumption and carbon dioxide production were determined by a metabolic

cart (Parvo Medics; Sandy, UT, USA). The highest achieved VO_2 value was reported as 'VO_2 Peak.' Blood pressure was manually measured using sphygmomanometer before, during each stage, and following the GXT to verify normal blood pressure responses to exercise and recovery.

Body Composition. Bone mineral density, lean mass, and percent body fat were determined using dual-energy X-ray absorptiometry (DXA; Hologic, Inc; Bedford, MA, USA). Body mass index (BMI) was calculated as body mass (kg) divided by height (meters) squared.

3.3 Cardiac Imaging

Cardiac imaging procedures were completed during one to two visits at UC Health Heart and Vascular Center at Medical Center of the Rockies, a multidisciplinary healthcare facility.

Echocardiography. Resting cardiac morphology was measured via echocardiography (EPIQ 7, Koninklijke Philips N.V., Amsterdam, NL) by a certified cardiac ultrasonographer. Images were collected during a single-breath hold over two to three cardiac cycles. Three-dimensional imaging was used to obtain accurate chamber volumes for the left ventricle (LV) and left atria (LA). 2-D imaging was used for length and volume for determination of additional two and four chamber measurements and LV area. Volumetric analysis was performed on DICOM images stored on IntelliSpace Cardiovascular workstation (EPIQ 7) using area and length measurements of the LV and LA and right atria. The Teichholz formula was then used to calculate ejection fraction (106) based on the volumetric data acquired. LV mass quantification and strain assessment were also determined using 2-D imaging. Doppler measurements were performed to evaluate systolic and diastolic function, as well as for assessment for wall motion abnormities and valve disease. All testing was completing in accordance with current professional guidelines (107).

Cardiac Computed Tomography (CT). Cardiac CT was used to generate coronary artery calcium scores (CACS) in a similar manner as described by others (1, 58, 63). Briefly, noncontrast-enhanced ECG-gated CT scans (Brilliance 64-Slice, Koninklijke Philips N.V., Amsterdam, NL) were completed using an average radiation dose of 0.9-1.4 mSv. Scans were conducted from the cranial to caudal direction using the following parameters: slice acquisition 40 x 0.26 mm, gantry rotation time of 500 ms, and a field of view of 22 cm. Images were collected during a single-breath hold and then saved off-line for CACS analysis. Calculation of CACS was performed by a licensed cardiologist blinded to the participant's exercise levels. CAC was detected according to density using Hounsfield units (HU). Individual calcified segments over the attenuation threshold of 130 Hounsfield units (HU) were identified. The Agaston Method was then used to determine CACS by multiplying the total area of each calcified region by 1, 2, 3, or 4, depending on the signal intensity. Multiplier values were assigned based on HU values, with 1 representing 130-199, 2 for 200-299, 3 for 300-399, and 4 > 400 HU (12). A composite score was then calculated using the individual scores of each calcified vessel. Each participant's CACS percentile was determined based on age and sex. Total CACS and individual lesion location were reported.

Cardiac Magnetic Resonance Imaging (MRI). Cardiac MRI scans were conducted to assess presence of myocardial fibrosis (MF). A standard assessment of chamber size, thickness, and contractility was performed (Achieva 1.5T, Koninklijke Philips N.V., Amsterdam, NL) with a multiplanar single-shot and cine imaging viability sequences. An intravenous infusion of 0.1 mmol/kg of MultiHance gadobenate dimeglumine (gadolinium) contrast was administered prior to imaging for fibrosis. Images for evaluation of MF were collected 9 min after contrast injection and 260-320 ms inversion times were used to attenuate the signal from normal

myocardium. Following gadolinium-enhanced imagining, a licensed radiologist blinded to the participant's training history analyzed the RV, RV outflow track, and LV for MF (Intellispace Portal, Koninklijke Philips N.V., Amsterdam, NL).

3.4 Vascular Function and Structure Assessments

Participants arrived to the laboratory at 7:00 am following a 12-h fast. Subjects were instructed to refrain from all exercise 24 hours before the study visit and strenuous exercise 48 hours before the study visit, avoidance of vitamins for 72 hours, as well as abstaining from use of all vasoactive medications. All premenopausal women were tested during the first phase of the uterine cycle. Some measures were not collected in all participants due to technical difficulties.

All vascular testing was performed in a dimly-lit, thermoneutral room (~20.0°C). Brachial blood pressures were measured in both the seated and supine position after ≥ 5 min of resting quietly. The arm was supported at heart level for all seated pressure measurements. Brachial pressure, calculated central (aortic pressures) and augmentation index (AIx %) were recorded using an automated cuff system (AtCor Medical, Inc.; Itasca, IL,USA).

Pulse Wave Velocity. After ≥ 20 minutes of rest in the supine position, carotid-femoral pulse-wave velocity (cfPWV), a measure of aortic stiffness, was assessed using a pencil-like tonometer probe and automated pressure cuff (AtCor Medical, Inc.; Itasca, IL,USA). Briefly, the investigator palpated for the left carotid pulse and femoral pulse. The location of the strongest carotid pulse was marked and a distance measurement was made from the mark to the suprasternal notch. Large calipers were used to measure the direct distance from the suprasternal notch to the pressure cuff wrapped around the proximal left thigh. Then, the distance from the leg cuff to the location at which the femoral pulse was detected was measured. Next, the carotid pulse wave form was detected at the marked location using the tonometer probe while the

femoral pulse was simultaneously monitored by the leg cuff. The elapsed time from the initial upstroke of the carotid pulse to the appearance of the femoral pulse was recorded. The distance measurements and the time between the pulse waves were used to calculate cfPWV using the subtraction approach to the foot-to-foot method.

Carotid Intima-Media Thickness and Compliance. Following cfPWV measurements, a longitudinal view of common carotid artery was continuously imaged proximal to the carotid bulb using an ultrasound linear array transducer 12 Hz probe (GE Healthcare; Wauwatosa, WI, USA). Simultaneously, the participant's cardiac cycle was monitored using a 3-lead ECG routed through the ultrasound machine. B-mode images were saved for offline analysis. Carotid intima-media thickness (CIMT) was defined as the distance between luminal edge of the vascular endothelium and the medial-adventitial border. Measurements were made on the far wall of the left common carotid artery using automated software designed to detect changes pixel-intensity (17) . Images chosen for analysis were taken during end-diastole between the P and Q waves in accordance with manufacturer recommendations.

B-mode images of the common carotid artery for carotid compliance were recorded offline (Medical Imaging Applications, LLC; Coralville, Iowa, USA) at 20 Hz. Cardiac cycle monitored by 3-lead ECG and central pressures determined by back calculation (AtCor Medical, Inc.; Itasca, IL, USA) were recorded during carotid imaging. Vessel diameter was defined as the distance between the luminal edge of the near wall intima to the luminal edge far wall intima. Change in vessel diameter from maximal systolic to minimal diastolic diameter with a pulse blood was used to calculated carotid compliance (83).

Vascular Compliance: $((\Delta D/D_D)/\Delta P*2))*\pi*D_D^2))$

D_D= Diastolic diameter ΔD= Systolic – Diastolic Diameter

Brachial Compliance. Brachial compliance was measured in a similar fashion to carotid compliance. A longitudinal view of the left brachial artery was visualized. Cardiac cycle and brachial pressure on the contralateral arm were measured during imaging. The measurements and calculations for carotid compliance described above were utilized during analysis.

Flow-Mediated Dilation. Vascular endothelial function was assessed using brachial artery flow-mediated dilation (FMD) based on established guidelines (109) . Following ≥ 30 min of rest in the supine position, a longitudinal view of the brachial artery was visualized. Once a clear image was obtained, probe position was refined to simultaneously acquire mean blood velocity while imaging the vessel in duplex mode. A rapidly-inflating cuff (D.E. Hokanson, Inc., WA, USA) was positioned immediately distal to the antecubital space to occlude forearm blood flow following baseline measurements. Brachial artery diameter and mean blood velocity were measured at rest and following 5 min of forearm ischemia. Brachial artery endothelial function was quantified as % Δ in brachial artery diameter = Peak diameter – baseline diameter / baseline diameter * 100. Brachial artery blood velocity was recorded and analyzed offline (DATAQ Instruments, OH, USA) and images were captured and then analyzed using automated edge-detection software (Medical Imaging Applications, IA, USA). Shear rate (SR) at rest and for each cardiac cycle after cuff deflation was calculated using the following formula SR= 8*(velocity/diameter) (77). Peak velocity following cuff deflation was reported as a measure of microvascular function. SR area under the curve (AUC) was calculated from cuff release to peak diameter using the Riemann sum technique to qualify the stimulus for FMD.

3.5 Venous Blood Samples

Venous blood samples were collected following > 8-h fast. Blood lipid panels were run to quantify total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol.

Lipoprotein a (Lpa), a subcomponent of LDL that is related to coronary heart disease (21), was also measured. A high-sensitivity analysis was performed to quantify C-reactive protein. Additionally, sex hormones testosterone and estradiol were measured. Thyroid function was evaluated based on circulating levels of thyroid-stimulating hormone (TSH). Complete blood counts (CBC) were also conducted to assess for potential anemia, platelet disorders, and abnormal immune cell values. All analyses were completed at UC Health Medical Laboratories (Medical Center of the Rockies Foundation, Loveland, CO and Poudre Valley Hospital, Fort Collins, CO) except for Lpa, which was determined by ARUP Laboratories (Salt Lake City, UT).

3.6 10-Year Cardiac Disease Risk

Cardiac Risk Calculator. 10-year coronary heart disease (CHD) risk was determined using a publically-available calculator (https://www.mesanhlbi.org/MESACHDRisk/MesaRiskScore/RiskScore.aspx, Supplemental Figure 2) developed from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort (57). This approach is valid for use in numerous racial/ethnic groups, and incorporates traditional risk factors as well as CACS obtained from cardiac CT imaging. Risk was evaluated using a minimum age of 45 based on the algorithm developed from the MESA data set. For participants age 40-44, 45 was used as the default age for risk calculations.

3.7 Statistical Analysis

All values are reported as means ± S.E.M. Comparisons of group characteristics and cardiac and vascular function and structure measurements were performed using unpaired t-tests for normally distributed variables (GraphPad Prism 7, La Jolla, CA). To assess for sex differences, a 2 x 2 analysis of variance (ANOVA) was used to determine sex by training status interactions. When appropriate, a Bonferroni's correction was applied for multiple comparisons.

When appropriate, CACS were compared between groups using categorical variables (CACS > 0 = "Yes," CACS < 0 = "No"). A X^2 Test was conducted to generate contingency tables with expected counts for the categorical analysis. When expected counts were <5, a Fisher's Exact Test was used. Statistical significance was set *a priori* at $p < 0.05$.

4. RESULTS

Participant characteristics are listed in Table 1**.** There were no significant differences in age or blood pressure between groups. As expected, ATH had lower % body fat, more weekly PA, greater aerobic fitness, and lower resting heart rate compared to CON.

Table 1. Participant characteristics. BMI, body mass index; MET-h, metabolic equivalent hours; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure**;** MAP, mean arterial blood pressure. Data are Avg ± SEM. * P < 0.05 vs CON.

Subject Characteristics	*CON*	*ATH*
n	18 (9M/9F)	25 (14M/11F)
Age, yrs.	49 ± 2	50 ± 1
BMI, kg/m^2	25.0 ± 0.9	21.6 ± 0.4*
Body Fat, %	31 ± 1.7	19 ± 1*
Physical Activity, h/wk	5.3 ± 0.5	11.5 ± 0.6*
Physical Activity, MET-h/wk	25 ± 3	123 ± 7*
Training history, yrs.	---	19 ± 2
VO_2 Peak, L/min	2.63 ± 0.20	3.47 ± 0.17*
VO_2 Peak, ml/kg/min	33.5 ± 1.6	53.0 ± 1.6*
Resting HR, bpm	53 ± 2	46 ± 1*
SBP, mmHg	120 ± 2	122 ± 2
DBP, mmHg	75 ± 2	75 ± 1
MAP, mmHg	90 ± 2	91 ± 1

4.1 Cardiac Morphology and Function

Comparisons of echocardiographic data revealed left ventricle (LV) mass tended to be higher in ATH than CON(171.1 ± 9.0 vs 137.4 ± 12.4 g, P = 0.06), while LV mass normalized to body surface area, and intraventricular septum (IVS) thickness were both significantly higher in ATH (Table 2). LV end diastolic volume (EDV) was also increased in ATH versus CON. Conversely, there was no difference in systolic or diastolic function based on ejection fraction and E wave to A wave (E:A) ratio, respectively.

Table 2. Cardiac structure and function determined by echocardiography (CON 5M/6 F, ATH 14M/10F). LV, left ventricle; IVS, intraventricular septum; EDV, end-diastolic volume; ESV, end-systolic volume; BP, biplane; RVDd, right ventricle diastolic diameter. * P < 0.05 vs CON.

Echocardiographic Data	*CON*	*ATH*
Cardiac Structure: Left Ventricle		Avg ± SEM
LV Mass, g	145.2 ± 12.4	170.4 ± 8.6
LV Mass, g/m^2	72.2 ± 4.7	92.9 ± 3.6*
IVS, cm	0.89 ± 0.05	0.97 ± 0.02
Posterior wall thickness, cm	0.91 ± 0.04	0.97 ± 0.03
EDV, mL	83.4 ± 6.5	107.5 ± 6.9*
EDV Index LV, mL/m^2	41.1 ± 1.8	62.4 ± 2.5*
ESV, mL	30.5 ± 3.6	41.1 ± 3.5
ESV Index LV, ml/m^2	14.7 ± 0.8	23.4 ± 1.4*
LV volume BP, mL	82.4 ± 4.8	114.7 ± 6.6*
Cardiac Structure: Right Ventricle		
RVDd base, cm	3.3 ± 0.1	4.1 ± 0.1*
RVDd mid, cm	2.3 ± 0.1	3.1 ± 0.1*
Cardiac Function		
Ejection Fraction, %	65 ± 1	64 ± 1
E:A ratio, AU	1.6 ± 0.2	1.5 ± 0.1

CACS generated from cardiac CT scans were available for 24 ATH (14M/10F) and 13 CON (6M/7F). Although 8 ATH (7M/1F) and 2 CON (2M) had CACS > 0, the presence of CAC was not different between groups (P > 0.05). CACS observed in ATH ranged from 6-510 while CACS in CON were 0.46 and 1.38. Percentiles assigned to the CACS based on age and sex for ATH ranged from 43rd to 96th and in CON percentiles were 26th and 41st. ATH had CAC in the left anterior descending (n=5), left circumflex (n=5), and right coronary arteries (n=3). In both CON with CACS > 0, the calcification was isolated to the right coronary artery.

Analysis of contrast-enhanced cardiac MRI to identify MF was completed for 24 ATH (13M/11F) and 8 CON (3M/5F). Neither ATH nor CON had any observable MF.

4.2 Vascular Function and Structure

Assessment of regional aortic vascular function demonstrated lower cfPWV values in ATH than CON (Figure 1) and in females than males (Figure 2), but there was no sex by training interaction. Similarly, local vascular function determined by carotid compliance revealed higher compliance in ATH compared to CON (Figure 3) and in female ATH than female CON (Figure 4). Additionally, there was no group difference in local compliance of the brachial artery (ATH; 9.4 x 10^{-3} vs CON; 8.1 x 10^{-3} mm^2/mmHg, $P > 0.05$). AIx was also similar between groups (Figure 5), although further analysis demonstrated a difference between male and female CON ($P < 0.01$, Figure 6). No differences were observed in vascular structure evaluated by CIMT (ATH; 0.64 ± 0.02 vs CON; 0.62 ± 0.03 mm, $P > 0.05$).

Vascular Endothelial Function. At rest, there was no difference in brachial artery diameter (Table 3) or velocity (data not shown). Following 5 min of forearm ischemia, peak diameter was achieved in a similar amount of time in both groups (Table 3), and there was no difference in absolute or percent change in diameter (Table 3, Figure 6). However, percent change in FMD was greater in female than male CON (Supplemental Figure 1). Additionally, microvascular function measured by peak velocity was not different between groups (Table 3). The shear stimulus quantified as SR_{AUC} was similar between groups (Table 3), and FMD normalized to SR_{AUC} was not different between ATH and CON (Table 3).

Table 3. Brachial artery FMD (CON 9M/9F, ATH 12M/10F).

Brachial Artery Characteristics	*CON*	*ATH*
Baseline Diameter, mm	3.20 ± 0.17	3.38 ± 0.11
Time to Peak Diameter, sec.	37 ± 3	37 ± 4
Av. Absolute Δ in Diameter, mm	0.16 ± 0.03	0.12 ± 0.04
Peak Velocity, cm/s	62.1 ± 3.3	58.8 ± 3.8
Shear $Rate_{AUC}$ /s · 10^3	19.8 ± 1.8	25.3 ± 4.0
Normalized FMD, (% Δ)/Shear $Rate_{AUC}$	2.5 · 10^{-4} ± 0.5 · 10^{-4}	1.6 · 10^{-4} ± 0.4 · 10^{-4}

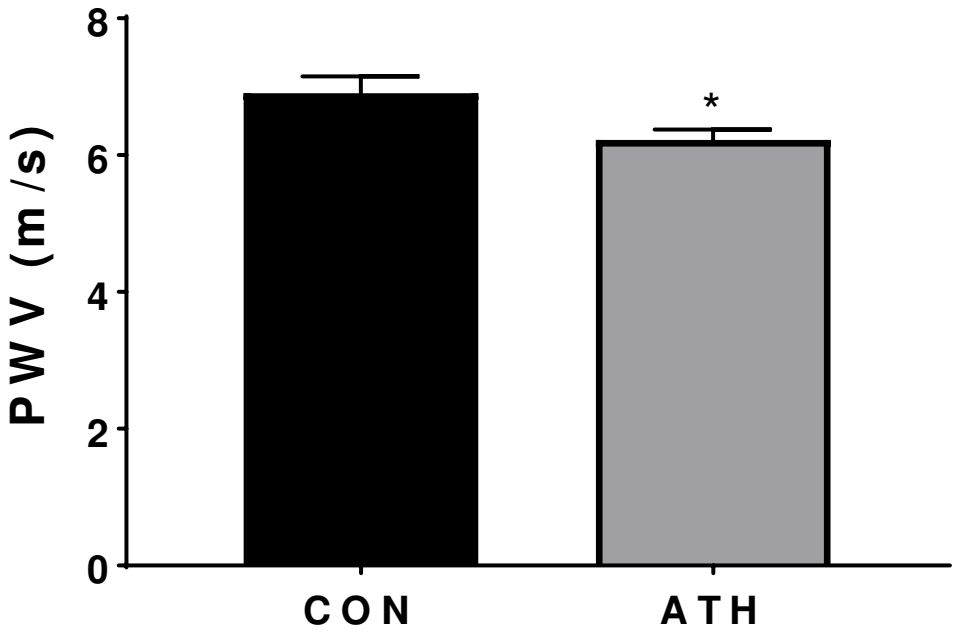


Figure 1. Vascular function assessed by cfPWV (CON 9M/9F, ATH 14M/11F). * P < 0.05 vs CON.

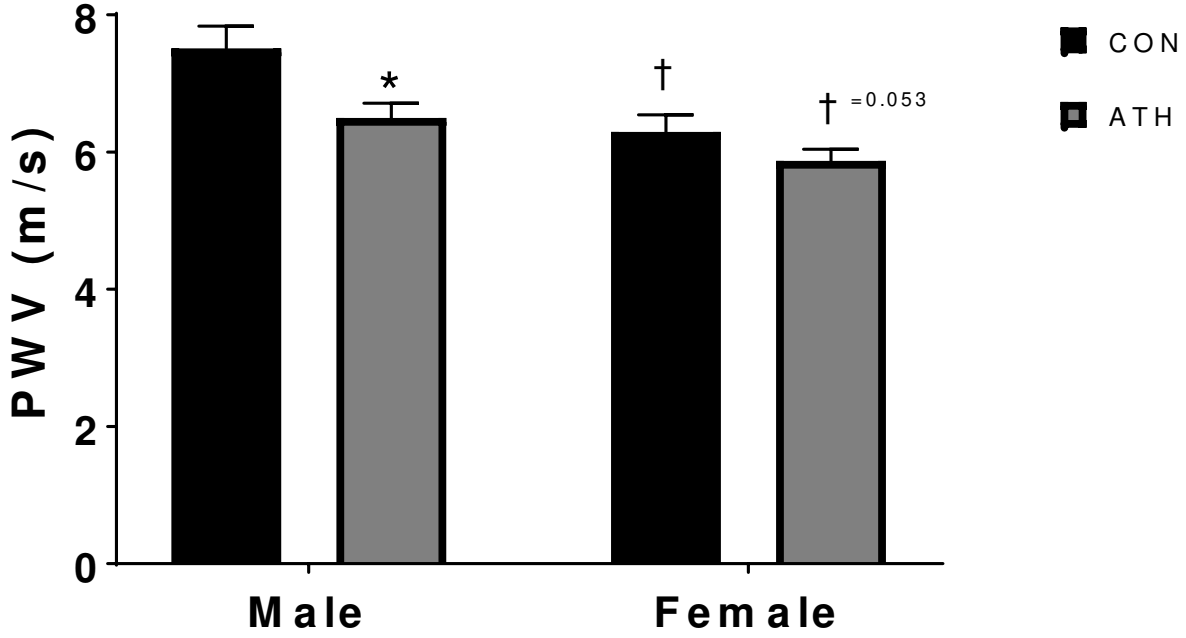


Figure 2. Vascular function assessed by cfPWV (CON 9M/9F, ATH 14M/11F).
* P < 0.05 vs CON within sex, † P < 0.05 vs M within training group.

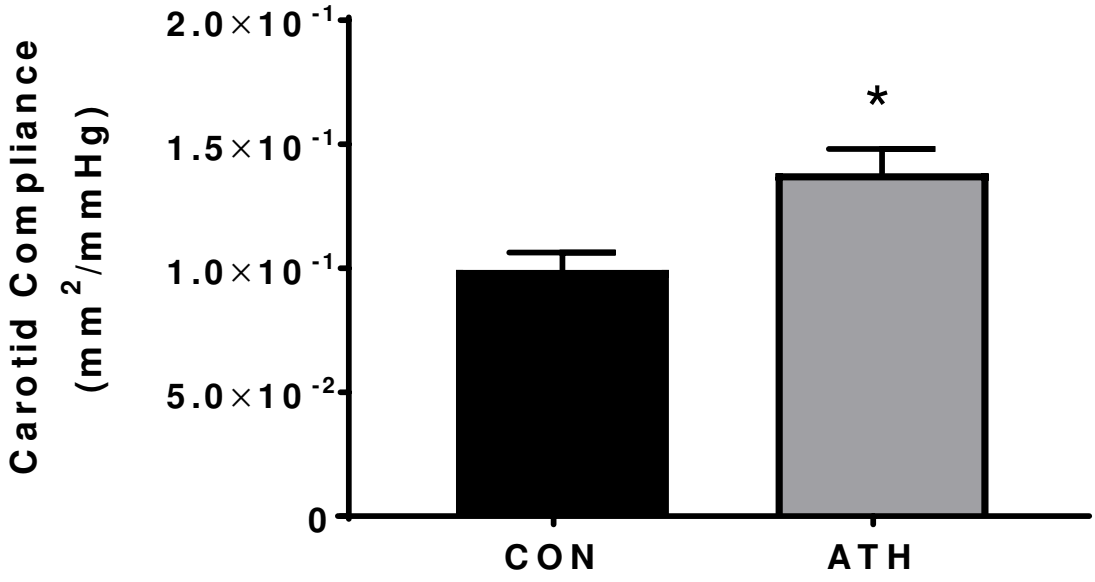


Figure 3. Vascular function assessed by carotid compliance (CON 9M/9F, ATH 12M/9F).
* P < 0.05 vs CON.

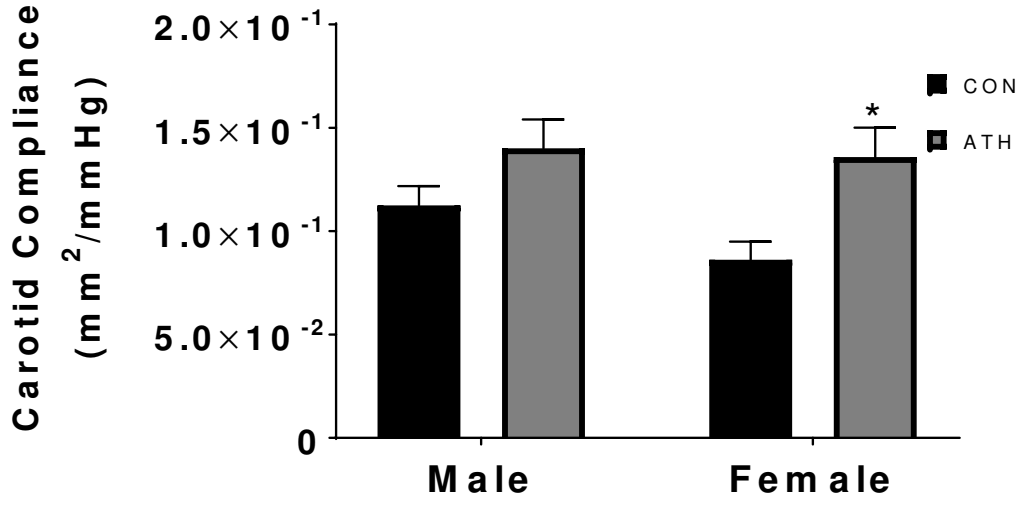


Figure 4. Vascular function assessed by carotid compliance (CON 9M/9F, ATH 12M/9F).
* P < 0.05 vs CON within sex.

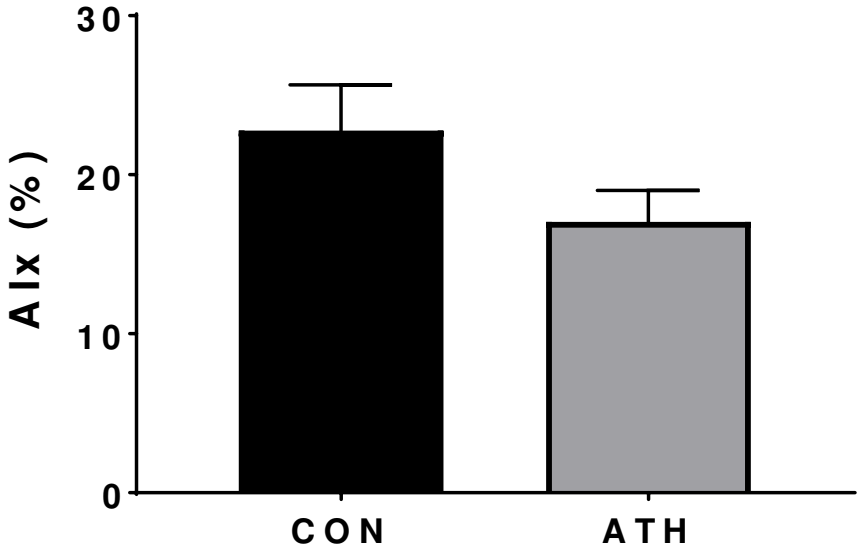


Figure 5. Vascular function assessed by AIx (CON 9M/9F, ATH 14M/11F).

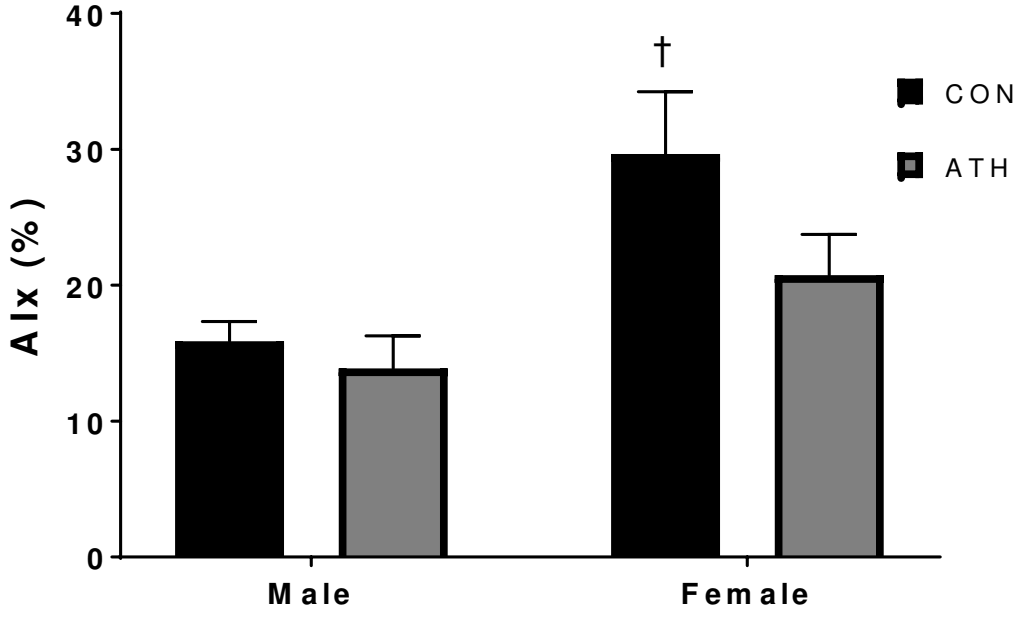


Figure 6. Vascular function assessed by AIx (CON 9M/9F, ATH 14M/11F).
† $P < 0.05$ vs M within training group.

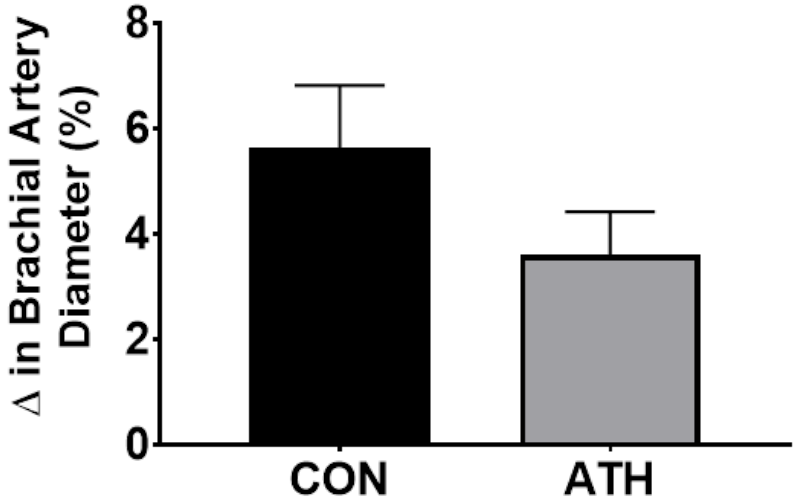


Figure 7. Vascular endothelial function assessed by brachial artery FMD (CON 9M/9F, ATH 12M/10F).

4.3 Venous Blood Samples

Venous blood sampling showed no differences between groups in circulating hormone levels (TSH, testosterone, estradiol; Table 4). Additionally, C-reactive protein, lipoprotein a as well as other lipoprotein measures (total, HDL, and LDL cholesterol) were similar between ATH and CON. However, triglycerides were lower in ATH compared to CON ($P < 0.01$) and ATH tended to have improved cholesterol ratio (total cholesterol:HDL), but this did not achieve statistical significance ($P = 0.07$).

Further analysis was performed to examine potential differences between male ATH with CACS > 0 (n=6) and CON (n=3). Similar to the full-group analysis, the subset of male ATH with CAC demonstrated lower circulating triglycerides (Supplemental Table 1, $P < 0.05$) compared to male CON. Male ATH with CAC also had higher amounts of HDL and reduced LDL (Supplemental Table 1) compared male CON. Furthermore, LDL:HDL and total cholesterol:HDL ratios were lower this subgroup of ATH than CON, indicating ATH had improved blood lipid profiles.

CBC testing showed similar values for RBC, Hb, Hct, and platelets between groups (Table 5). Conversely, WBC (Table 5), mean corpuscle [Hb], and absolute neutrophil, monocyte, and eosinophil levels were different between groups (Supplemental Table 2). Nonetheless, all values were within the normal ranges. Additional CBC parameters are listed in Supplemental Table 2.

Table 4. Venous blood samples (CON 5M/7F, ATH 13M/10F). * P < 0.05 vs CON.

Venous Blood Samples	*CON*	*ATH*
		Avg ± SEM
Thyroid stimulating hormone, mIU/L	2.3 ± 0.5	2.3 ± 0.4
Testosterone, ng/dL	166 ± 59	226 ± 46
Estradiol, pg/mL	43 ± 10	43 ± 17
C-reative protein, mg/L	1.64 ± 0.54	1.49 ± 0.65
Lipoprotein a, mg/dL	28 ± 8	31 ± 11
Triglycerides, mg/dL	115 ± 12	72 ± 6*
Total cholesterol, mg/dL	210 ± 10	197 ± 6
HDL cholesterol, mg/dL	63 ± 6	75 ± 3
LDL cholesterol, md/dL	123 ± 11	107 ± 6
LDL : HDL ratio, AU	2.22 ± 0.30	1.51 ± 0.12*
Total Chol : HDL ratio, AU	3.63 ± 0.35	2.72 ± 0.14*

Table 5. Complete blood count (CBC) data from venous blood samples (CON 5M/7F, ATH 13M/10 F). RBC, red blood cell; WBC, white blood cell; Hb, hemoglobin; Hct, hematocrit. * P < 0.05 vs CON.

Complete Blood Count	*CON*	*ATH*
		Avg ± SEM
RBC, 10^{12}/L	4.92 ± 0.16	4.81 ± 0.09
WBC, 10^{9}/L	6.0 ± 0.4	4.8 ± 0.2*
Hb, g/dL	14.7 ± 0.6	14.8 ± 0.2
Hct, %	43.7 ± 1.4	43.4 ± 0.7
Platelete Count, 10^{9}/L	231 ± 9	223 ± 10

4.4 Cardiac Risk

10-year CHD risk was calculated using MESA Risk calculator for 22 ATH (12M/10F) and 12 CON (5M/7F). No group differences were detected in 10-year cardiac event risk (ATH; 2.4 ± 0.6 vs CON; 1.6 ± 0.3 %, P > 0.05).

5. DISCUSSION

To our knowledge, this is the first time both cardiac and vascular measures have been made in a single group of subjects to examine the influence of long-term (> 10 years) ultra-endurance training on cardiovascular risk. The primary findings in this study were 7 male ATH has CACS > 0 compared to 2 male CON, although these differences did not reach statistical significance. Conversely, only 1 female ATH had a CACS > 0 and no female CON had any detectable CAC. Surprisingly, no MF was observed in any participants, and measures of vascular function that are associated with CVD risk (FMD and cfPWV) were not impaired in the ATH group. Furthermore, cfPWV was lower and carotid compliance was higher in the ATH group, indicating ATH had improved regional and local vascular compliance compared to CON. These data indicate that although some ATH have CAC, overall, the ATH do not have an elevated risk of developing CVD compared to adults that meet current physical activity guidelines.

5.1 Coronary Artery Calcium

CACS is related to 10-year CVD event risk and is considered a clinical marker of atherosclerosis (56, 57). However, CAC can develop in both the arterial intima and media, and the location of the calcification cannot be determined by cardiac CT. The inability to ascertain exactly where the calcium deposits reside makes it difficult to distinguish between atherosclerotic calcification and CAC not resulting from coronary plaque deposition. In the current study, 7 male ATH and only 1 female ATH had CAC, while 2 males and no female CON had any detectable calcification. Previous investigations (1, 14, 58, 63, 84, 85) have also reported elevated levels of CAC in masters athletes, and much of these studies have focused on

males (1, 14, 63, 84). However, a recent larger study reported CACS in both middle-aged (~50 y) trained males and females (58). Dissimilar to the current study, the authors did find CAC in multiple female athletes (n=10), but there was no difference in median or percentile calcium scores between women runners and cyclists compared to sedentary controls- suggesting that the trained females do not have a great likelihood of developing CAC compared to sedentary controls. Additionally, Roberts et al. (2017) studied exclusively female marathon runners and found the runners had less CAC than controls. Nonetheless, both groups in this study had some amounts of CAC and the control participants were obese and had a greater history of hypertension, dyslipidemia, smoking, and family history of coronary artery disease, calling in to question the appropriateness of using those individuals for comparison. Surprisingly, blood lipid profiles (TG, HDL, LDL:HDL, Total Chol:HDL, Supplemental Table 1) in male ATH with CACS > 0 in our study were more favorable compared to male CON ($p < 0.05$). Additionally, no participants in either group had a history of diabetes, hypertension, smoking, or other chronic disease. We expected that increased CACS in ATH is related in part to these risk factors since other studies (1, 14, 63, 84, 85) have not excluded participants based on those characteristics. The one female participant with CAC did have family history of premature cardiac disease, but the potential interaction between endurance training and inheritance of coronary artery disease cannot be determined from this information.

When examining CACS distribution amongst the general population, it does not appear that our small sample exhibits greater average CACS than those in the general population (37 vs 121) who do not engage in heavy endurance training (43). However, it is unclear when CAC first appears in ATH and little work has been done to see if CAC in ATH is related to increased incidence of cardiac events (63, 64). Others have speculated that CAC in ATH may actually be

protective due to a stabilizing effect of calcification of soft plaque (12, 26). However, calcified plaque that contains a lipid core may still have the potential for rupture compared to a more homogenous calcified lesion (20). Improvements in calcium scoring approaches including measures of calcium density may be more valuable in this unique athletic population compared to using traditional scores for the general population (12). Importantly, two ATH (1 male, 1 female) had high total and percentile CACS (510, 96th percentile and 268, 86th percentile, respectively). These scores appeared to be much greater than the other calcium scores observed (6-30) amongst male ATH and CON in this study. Considering calcium scores do correlate with total plaque volume (88) and higher CACS are more indicative of plaque than lower scores (56), it is possible that the higher scores are more related to an atherosclerotic process while the lower amounts of calcium develop in a different manner that is primarily related to exercise. It is important to note, there is not sufficient evidence at this point in time to support this claim and more work is needed to distinguish the potential different etiologies of CAC formation within endurance trained middle-aged adults.

5.2 Myocardial Fibrosis

The fact that no ATH participants exhibited MF creates doubt that fibrosis is in fact directly related to long-term ultra-endurance training. It has been suggested that MF in ATH is related chronic pressure overloading, and elevated pulmonary artery pressures due to much greater sustained cardiac outputs (CO) achieved in endurance-trained individuals may lead to more fibrotic development within the right ventricle (RV) versus the left (41). Furthermore, extrapolated pulmonary pressures resulting from high CO may reach pressures observed in pulmonary artery hypertension (PAH) patients (41, 87). Not surprisingly, these PAH patients exhibit MF that tends to be specific to the RV. To support this notion, La Gerche and colleagues

(2012) observed impairment of RV ejection fraction and fractional area change immediately following a 3-11 hour endurance events and RV ejection fraction was lower in athletes that exhibited interventricular septum fibrosis. Additionally, multiple studies (8, 91) using rodent models of endurance training indicate fibrotic development is more pronounced amongst rats trained at high intensity (~85% of VO_2 max for 1hr., 5 days/wk) versus a moderate intensity (~60% of VO_2 max for 45 min., 5 days/wk). These data suggest that the combination of high intensity and longer duration aerobic exercise leads to maladaptive fibrotic deposits in the myocardium.

We hypothesized that ATH would have greater amounts of fibrosis than CON based on the data described above. As already mentioned, we found no fibrosis in any ATH, indicating the current study appears to contradict other investigations that have examined MF in ATH. However, we believe that the presence of MF in ATH may not be related to training, and fibrosis may develop in some chronically-trained individuals due to non-exercise related causes. For instance, a number of investigations that have measured MF (58, 94) have shown a variety of patterns of fibrosis. Some of these patterns are consistent with previous myocardial injury due to ischemic events and infection or other cardiomyopathy. The studies that describe in MF in ATH also tend to be case studies (94) or have larger sample sizes (58). Surprisingly, one study that examined 12 life-long (43 years of training) middle-age male athletes found 50% of participants to have some fibrosis (122), while a larger study only detected fibrosis using gadolinium contrast in 15 out of 106 male athletes. When examining thirteen other studies with small to moderate numbers of trained participants (n= ~20-100), presence of MF tends to range from 0-13%, with more than half of those studies reporting no detection of fibrosis (94). This would suggest that our study may not have been powered to detect MF, which may indicate that this measure is not

strongly associated with exercise training. Additionally, the proposed relationship between pulmonary artery pressure and RV pressure overload has not been directly observed, leaving this claim unsubstantiated.

5.3 Carotid-femoral Pulse Wave Velocity

We chose to use cfPWV as a method of evaluating vascular function because of the predictive ability of this measure to determine CVD event risk (7, 60, 101). Historically, exercise training has been shown to be related to increased vascular compliance (18, 65, 104), thereby decreasing vascular stiffness. Accordingly, cfPWV tends to be lower in older athletes compared to sedentary subjects (114). Although resistance training can negatively impact vascular compliance (62), little evidence exists (115) to indicate that endurance training is related to vascular stiffening, thus potentially elevating CVD event risk. Nonetheless, one investigation did find marathon runners to have higher cfPWV compared to controls (115), and, due to the context of our research question, we thought that high-volumes of training may negatively affect aortic compliance compared to the more moderate volumes of exercise required to achieve beneficial responses in other vascular beds. In contrast to our hypothesis, cfPWV was lower in ATH, indicating greater compliance. Similarly, carotid compliance was also enhanced in ATH compared to CON and augmentation index (AIx), a measure of microvascular stiffness, tended to be lower in ATH ($p = .096$). Collectively, these data indicate positive central and peripheral vascular adaptations related to training status.

A seemingly disparate finding was the lack of difference in brachial artery compliance between groups. However, vascular media composition transitions from greater elastin dominance centrally to more muscular phenotype peripherally (39). Moreover, central elastic vessels like the aorta are more effected by age-related stiffening than peripheral muscular vessels

(42, 97). Based on these data, we can only speculate that there may be a differential training effect between large elastic and muscular arteries, where exercise can improve or offset age-related increases in arterial stiffness only in the affected elastic beds.

5.4 Flow-Mediated Dilation

Another measure of vascular function we collected was FMD. Specifically, FMD is a non-invasive means of assessing vascular endothelial function, thus adding to our integrative approach of assessing vascular function in the current study. Importantly, large population-based studies reveal that diminished FMD is related to CVD event risk (95, 124, 125) and lower FMD values reported as a percent change in brachial artery diameter have been observed in various clinical populations (31, 53, 72, 117). For this reason, we decided to measure FMD in the current study in addition to other cardiac and vascular measures to evaluate CV risk. Furthermore, brachial artery FMD has been correlated with coronary artery endothelial function (4, 53, 72), so we expected that ATH with CAC would also have reduced FMD compared to CON participants. Surprisingly, there was no difference in FMD between groups, suggesting ATH did not have impaired vascular endothelial function. Nonetheless, it has been proposed that a change in FMD over time is more meaningful than an individual measurement at a single point in time (37), which is an important consideration for future longitudinal studies.

A number of publications demonstrate in both cross-sectional and interventional studies that physical activity improves FMD (9–11, 25, 27, 74, 80, 82, 86), although some data show FMD is not enhanced in trained individuals or following an exercise intervention (38, 69, 80, 90). This apparent discrepancy raises questions about the usefulness of FMD to appraise CVD risk in active or athletic populations. FMD appears to be a valuable tool in part due to the reliance of this response on nitric oxide (NO, ~70% of dilation response) (34). For this reason,

some believe that evaluation of FMD is a non-invasive means to determine NO availability, which is important due to its antithrombotic and anti-inflammatory properties that can retard the progression of atherosclerosis (116). Nonetheless, methodological differences, variability of NO contribution to FMD between different vascular beds (109), and the impact of baseline artery diameter on percent change (108, 111) reveal that single FMD values without situational context limit the conclusions about vascular health that can be drawn about the presence or lack of group differences in FMD based on a single measurement. Currently, it is unclear if lower FMD values in ATH do actually represent vascular dysfunction and if exercise has the paradoxical influence of improving FMD at lower volumes, but not enhancing the FMD response when individuals engage in higher training volumes.

Some data indicate sex differences in the FMD response to training (69, 80, 90), and this divergence is likely influenced by endogenous sex hormones (35, 69). Many studies have tried to circumvent this issue by dividing women into distinct pre and post-menopausal groups (10, 69, 80, 89) or by excluding studying women altogether (9, 79, 117, 118). In the current study, we acknowledge the obvious gap in knowledge created by deciding not to study women throughout the menopause transition in their 40s and early 50s. In an attempt to account for hormonal changes, we matched ATH and CON for menopausal status based on participant interviews and verified reported menopausal status using plasma measures of estradiol. Furthermore, we tested all premenopausal women during the first phase of the uterine cycle in accordance with current guidelines (109) to try to control for monthly hormone fluctuations. Typically, women have improved FMD compared to men until menopause (16) when estrogen bioavailability begins to fall, then values tend to be comparable between sexes (45). Surprisingly, our female CON group had greater FMD compared to CON men, while this

difference was not observed with ATH (Supplemental Figure 1). Therefore, we cannot rule out the effect of endogenous sex hormones on this measure. Nonetheless, recent data in young healthy women that conflicts with the belief that estrogen has a major influence on FMD (68, 120) shows that monthly hormonal variations do not impact brachial artery FMD (96). This inconsistency reveals much is still unknown in regard to the relationship between vascular endothelial function and age, hormonal status, and exercise training in women.

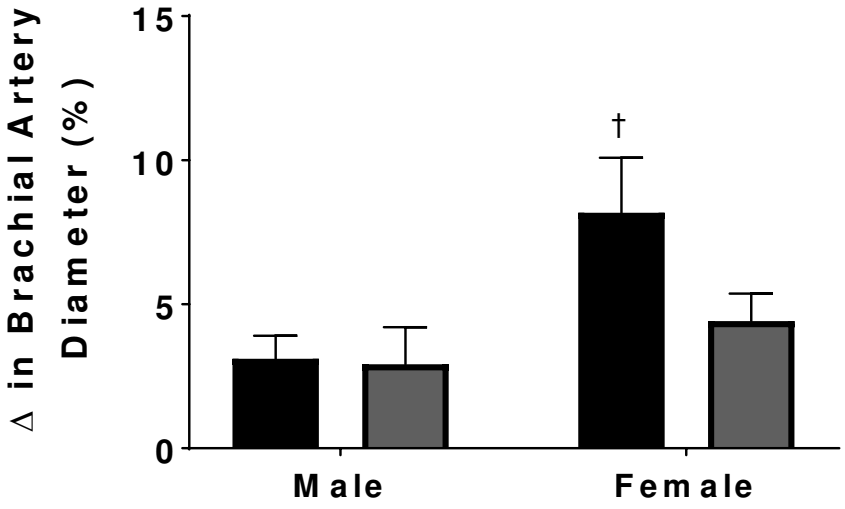
5.5 Perspectives

The overall goal of this study was to evaluate cardiac and vascular structure and function in middle-aged men and women training for ultra-endurance events and active controls to determine the influence of exercise volume on cardiac risk. We chose a weekly exercise volume of ≥ 100 MET in an attempt to substantially surpass the training volumes of individuals examined in other studies of similar design (1, 63). Using clinically relevant markers of CVD risk (BP, total cholesterol, HDL cholesterol) along with our CACS and other participant characteristics, we used a risk calculator (57) to determine 10-year CHD risk. Despite the presence of CAC in some ATH, we did not observe group differences in 10-year risk. Additionally, no ATH with a CACS > 0 were at high 10-year event risk (> 20%) based on risk stratification categories (54). Furthermore, carotid intima-media thickness (CIMT), a vascular structure measure related to CV event risk (55, 76) was not different between ATH and CON.

In an effort to gain a comprehensive understanding of cardiovascular structure and function in the ATH, we utilized numerous techniques for imaging the heart as well taking a variety of measurements along the vascular tree. Echocardiographic data revealed structural differences between CON and ATH (e.g., LV mass index, EDV; Table 2) consistent with training adaptations while ejection fraction and E:A ratio were similar, indicating cardiac

morphology in the ATH group did not accompany impaired function. As described above, we did not observe any MF, which is often accompanied by tachycardic arrhythmias and diastolic dysfunction (23). The lack of MF in any ATH further indicates that these individuals may not be at an elevated risk of sudden cardiac death due to an arrhythmic event compared to CON. Although both groups demonstrated low values of cfPWV, ATH still had greater aortic compliance than CON. Considering cfPWV related to risk, perhaps ATH have some risk reduction related to this aspect of vascular function. Moreover, our risk calculations do not incorporate vascular factors such as cfPWV. The risk factor gap suggests that vascular adaptations to exercise training may be responsible for the risk reduction beyond changes in traditional risk factors. Therefore, the ATH studied in the current study may actually have lower CV event risk despite some having CAC, but this could not be determined by the risk calculation method we used. Collectively, these data suggest that the training performed by ATH group is not contributing to a higher risk of experiencing a cardiac event since exercise volume was the primary demographic difference between the ATH and CON groups.

6. SUPPLEMENTAL DATA



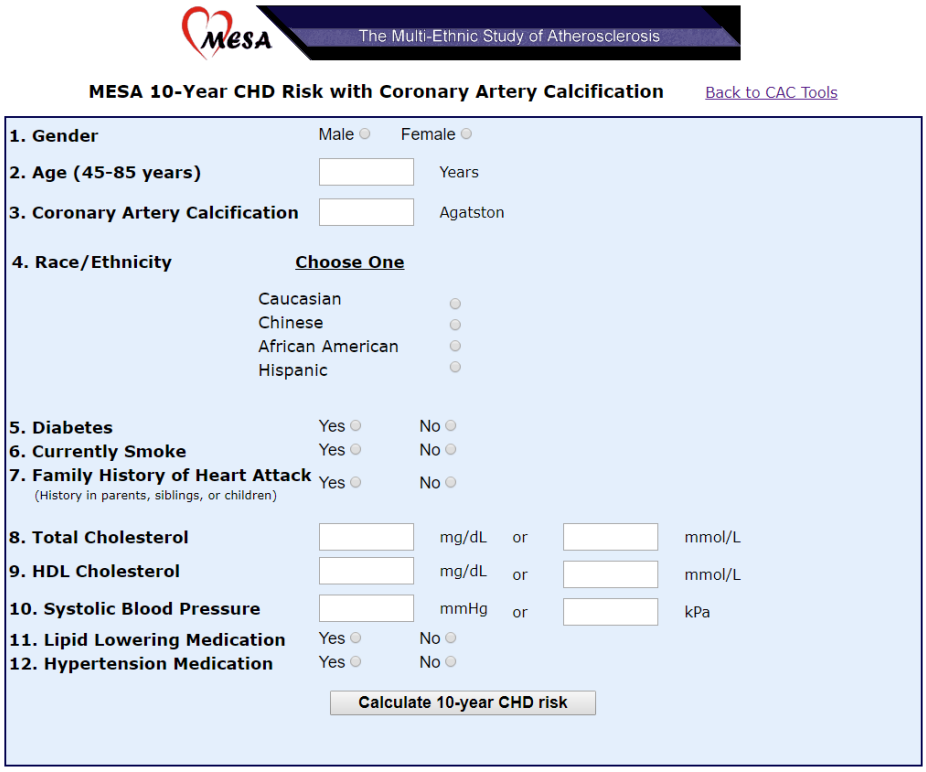
Supplemental Figure 1. Vascular endothelia function assessed by brachial artery FMD.
† P < 0.05 vs M within training group.

Supplemental Table 1. Male venous blood samples (CON 5, ATH 6). * P < 0.05 vs CON.

Venous Blood Samples	*CON*	*ATH*
		Avg ± SEM
Triglycerides, mg/dL	135 ± 13	85 ± 12*
Total cholesterol, mg/dL	222 ± 15	212 ± 10
HDL cholesterol, mg/dL	46 ± 3	67 ± 4*
LDL cholesterol, md/dL	148 ± 11	127 ± 8
LDL : HDL ratio, AU	3.23 ± 0.21	1.94 ± 0.16*
Total Chol : HDL ratio, AU	4.83 ± 0.25	3.21 ± 0.20*

Supplemental Table 2. Additional complete blood count (CBC) parameters from venous blood samples (CON 5 M/ 7 F, ATH 13 M/ 10 F). * P < 0.05 vs CON.

Complete Blood Count	*CON*	*ATH*
		Avg ± SEM
Mean Corpuscular Vol., 10^{-15} L	89.0 ± 1.9	90.9 ± 0.8
Mean Corpuscular Hb, pg	30.0 ± 0.9	31.0 ± 0.3
Mean Corpuscular [Hb], g/dL	33.6 ± 0.5	34.1 ± 0.1*
Mean Platelete Vol., 10^{-15} L	9.8 ± 0.2	9.8 ± 0.2
Red Cell Distribution Width, %	13.2 ± 0.4	12.6 ± 0.1
Neutrophils Absolute, 10^9/L	3.5 ± 0.4	2.7 ± 02*
Lymphocyte Absolute, 10^9/L	1.8 ± 0.1	1.6 ± 0.1
Monocytes Absolute, 10^9/L	0.5 ± 0.03	0.4 ± 0.02*
Eosinophils Absolute, 10^9/L	0.2 ± 0.03	0.1 ± 0.01*
Basophils Absolute, 10^9/L	0.04 ± 0.01	0.03 ± 0.01



Supplemental Figure 2. MESA 10-Year Risk Calculator.

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