EFFECTS OF CHANGES IN WEIGHT, BODY COMPOSITION, FITNESS AND PHYSICAL ACTIVITY ON AORTIC WAVE VELOCITY IN OVERWEIGHT AND OBESE ADULTS

by

Anne Marie Moody

B.S. High Point University, 2009

M.S. Appalachian State University, 2011

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by

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Emma Barinas-Mitchell, Assistant Professor, Epidemiology
Fredric L. Goss, Associate Professor, Health & Physical Activity
John M. Jakicic, Full Professor, Health & Physical Activity
Dissertation Advisor: Bethany Barone Gibbs, Assistant Professor, Health & Physical Activity
Vascular adaptations, such as arterial stiffness, have been established as precursors to cardiovascular outcomes, such as hypertension, stroke, and heart failure. Arterial stiffness of the aorta is elevated in conditions of obesity, specifically abdominal obesity, physical inactivity, and low cardiorespiratory fitness. Standard behavioral weight loss interventions often promote weight management through decreased caloric intake and increased physical activity. Whether increased physical activity and related gains in fitness have independent or additive effects with weight loss on aortic stiffness remains unclear. **PURPOSE:** The purpose of this study is to determine the overall and independent effects of changes in overall and abdominal obesity, fitness, and physical activity within a standard behavioral weight loss intervention on carotid-femoral pulse wave velocity. **METHODS:** Eighty three (N=83) overweight and obese adults (age 44.5 ± 8.1 years; BMI 32.4 ± 4.0 kg/m²) completed 6-months of a standard behavioral weight loss intervention. Aortic, or, carotid-femoral pulse wave velocity (cfPWV) was assessed by applanation tonometry, abdominal obesity by dual X-ray absorptiometry (DXA), objective moderate-vigorous physical activity (MVPA) by BodyMedia SenseWearPro armband (Pittsburgh, PA) and cardiorespiratory fitness by graded exercise testing. **RESULTS:** After 6 months, cfPWV, body weight, BMI, waist circumference, total body fat, and abdominal fat significantly decreased, while VO2(85%MHR) significantly increased (all \( p<0.000 \)).
VO2(85%MHR) marginally predicted change in cfPWV, independent of changes in body weight and abdominal fat ($p=0.045$). Changes in MVPA variables were not predictive of changes in cfPWV (all $p>0.1$). Changes in body weight, abdominal fat, and all other obesity variables were not associated with changes in cfPWV (all $p<0.1$) **CONCLUSIONS:** These findings indicate that improvements in fitness, and not adiposity, were important for decreasing cfPWV. These results highlight the potential importance of targeting fitness in lifestyle interventions to reduce the elevated cardiovascular risk observed in sedentary and overweight or obese populations.
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1.0 INTRODUCTION

1.1 CARDIOVASCULAR DISEASE AND ARTERIAL STIFFNESS

Cardiovascular disease affects 26.5 million American men and women and is the leading cause of morbidity and mortality in the United States.\textsuperscript{1} Traditional risk factors for cardiovascular disease include hypertension, dyslipidemia, glucose dysfunction and obesity, remain highly prevalent and are well known to contribute to the burden of disease.\textsuperscript{2,3} More recently, vascular adaptations such as arterial stiffness and endothelial dysfunction have been established as independent predictors for cardiovascular risk.\textsuperscript{4} Changes in vascular structure and function are thought to be early manifestations of future health outcomes, such as hypertension, stroke and ventricular heart failure.\textsuperscript{5}

Arterial stiffness is characterized by structural adaptations of the arterial system that occur as part of the normal aging process. Elastin fibers are gradually replaced with more rigid collagen fibers and this change is accompanied by increased glycalated end products, increased growth factors and vascular smooth muscle cell hypertrophy yielding a stiffer artery.\textsuperscript{5} The complexity of these mechanisms is still not fully understood, but appears to manifest in a separate pathway from atherosclerotic disease.\textsuperscript{6} This pathophysiology can occur throughout the entire vasculature, but increased stiffness of central arteries (i.e. aortic stiffness) is more
consistently associated with cardiovascular outcomes when compared to peripheral artery stiffness.\textsuperscript{7}

Aortic stiffness can be measured by a variety of techniques, varying in cost, technical skill required, and clinical application. Pulse pressure, pulse wave velocity (PWV), ultrasound, magnetic resonance imaging (MRI), and waveform analysis are among the most commonly used in clinical practice or research.\textsuperscript{8} Of these, PWV is considered the gold standard for arterial stiffness assessment and the measurement has been well validated.\textsuperscript{9} PWV captures the transit time of a pressure waveform that travels through the arterial branches following an ejection of blood from the heart. Carotid-femoral pulse wave velocity (cfPWV), also called central or aortic PWV, is assessed by capturing waveforms at the superficial carotid and femoral arterial points via piezoelectric sensors. A slower velocity is indicative of a healthier and more compliant artery, while a faster velocity reflects stiffer arteries.

\subsection*{1.2 ARTERIAL STIFFNESS AND ASSOCIATED RISK FACTORS}

Consistent relationships have been observed between cfPWV and CVD risk factors and cardiovascular outcomes.\textsuperscript{10-13} Longitudinal evidence reveals higher cfPWV as a precursor to more chronic vascular deficiencies, specifically hypertension\textsuperscript{14-16} which affects one in three adults.\textsuperscript{1} Early detection of elevated cfPWV could be beneficial in preventing hypertension and progression to ventricular hypertrophy and heart failure.\textsuperscript{17} It is estimated for each one meter per second (m/s) higher cfPWV, cardiovascular disease and mortality risk is 14-15\% greater.\textsuperscript{18} Therefore, understanding the impact of reducing modifiable risk factors that, in turn, relate to arterial stiffness may help attenuate declines in arterial health. For the proposed study, the risk
factors of interest are overall obesity, abdominal obesity, cardiorespiratory fitness (CRF) and moderate-vigorous physical activity (MVPA).

1.2.1 Overall and Abdominal Obesity

The 2011 National Health and Nutrition Examination Survey (NHANES) estimates that 34.9% of Americans are obese. Obesity is a chronic metabolic disorder that is well established as a risk factor for cardiovascular morbidity and mortality. Each 5 kg/m² increase in body mass index (BMI) above 25 kg/m² is estimated to increase cardiovascular mortality by 40% and overall mortality by 30%. In addition, higher cfPWV is observed in obese individuals with one study revealing median cfPWV values to be 14% higher in obese compared to nonobese.

The risk of heart failure also increases across higher BMI categories and compared with normal weight, obese subjects have twice the risk of heart failure.

Obesity is well-known to cause remodeling of vascular structure and hemodynamic function as a result of greater blood volume, increased cardiac workload, and increased sympathetic activation. Chronic stress on the arterial wall prolongs the systolic phase, reduces vessel compliance and eventually manifests as arterial stiffness. Such changes have direct implications on future risks of cardiac insufficiency, heart failure and mortality. Obesity likely contributes to higher cfPWV through various other mechanisms including excessive lipids, proinflammatory factors and insulin resistance. In addition to a primary role of fat storage, adipose tissue is recognized as an inflammatory organ and, in excess, releases proinflammatory cytokines, typically referred to as adipokines. Weight gain is accompanied by adipocyte hypertrophy and additional proinflammatory factors that exacerbate inflammatory damage to the vasculature. As these adipocytes exceed their normal storage capacity and accumulate in other
tissues, normal metabolic and hemodynamic function is disrupted, typically presenting as insulin resistance and sympathetic dominance. A timeline of mechanistic events has not yet been identified, but it does appear that each action exacerbates the other.

Studies of the relationship between overall obesity, or total body fat, and aortic stiffness have produced mixed results across age groups. There appears to be an age-dependent effect where higher body fat is associated with lower cfPWV in young adults but becomes associated with higher cfPWV after the age of 50. In middle-aged adults, several longitudinal studies have reported weak associations between obesity and cfPWV. Weight change has been shown to influence arterial stiffness; weight gain has been associated with the progression of arterial stiffness, while weight loss has been shown to regress stiffening. Experimental studies with standard behavioral weight loss interventions (SBWL) that have produced modest weight losses (5-10% of baseline body weight) have shown improvements in arterial stiffness measures independent of other obesity-related factors, and weight change has been associated with the change in cfPWV. Further assessment of overall obesity and cfPWV at baseline and over time would be valuable in SBWL, specifically within a middle-aged population.

Abdominal obesity is characterized by excessive abdominal fat and is highly related to cardiovascular outcomes independent of overall obesity. Some researchers have found independent associations between abdominal obesity and cardiovascular risk, while others believe the relationship is mediated by other risk factors such as hypertension. A higher proportion of abdominal fat corresponds with a higher cfPWV in obese and nonobese individuals, persists across age groups and even modest abdominal fat gains significantly worsen measures of stiffness. These associations are present independent of total body weight or body mass index (BMI) and with both visceral and subcutaneous trunk
adiposity. To this author’s knowledge only one experimental study has investigated the effect of abdominal weight loss on arterial stiffness using sophisticated measurements of abdominal obesity (e.g. dual energy x-ray absorptiometry (DXA)). Similar assessment in longer-term SBWL with these gold standard measurements, greater sample size and longer follow-up is warranted to further clarify the role of reducing overall and abdominal adiposity on cfPWV in a healthy, obese population.

The mechanisms linking abdominal fat to arterial stiffness independently from overall obesity are not well understood, but may partly be explained by strain on the arterial wall resulting from a chronic proinflammatory state. However, experimental evidence linking changes in abdominal obesity to changes in cfPWV in healthy obese individuals is lacking because most studies have small sample size, are short-term and use suboptimal methods of abdominal obesity assessment (e.g. waist circumference). Further exploration of the independent relationship between changes in abdominal obesity and arterial stiffness is needed to elucidate this potential pathway for improving vascular health.

1.2.2 Cardiorespiratory Fitness and Moderate-Vigorous Physical Activity

Low CRF and a lack of MVPA are well-established risk factors for cardiovascular disease. Physical activity encompasses 2 main components: 1) exercise that is planned, structured activity and 2) non-exercise activity that may include occupational, recreational, or household activity, although research is often limited to a specific component of MVPA (e.g. exercise or occupational activity). More specifically, MVPA is used to describe activities which encompass some amount of both moderate and vigorous intensities, and these can be measured as metabolic cost (MET) or as a percentage of age-predicted maximal heart rate or oxygen consumption.
Though related, CRF is distinct from MVPA in that it is the physiological response of body systems to supply oxygen during a given activity. Incidence of cardiovascular disease is higher in individuals with lower CRF and lower MVPA levels, justifying the importance of targeting these outcomes in intervention studies. Furthermore, data suggests that habitual aerobic exercise can delay the age-related increase in arterial stiffness, which may benefit future cardiovascular outcomes.

Higher CRF and MVPA levels improve arterial stiffness through both structural and functional adaptations on the vessels. Cross-linking of collagen and elastin fibers is the primary structural contribution to arterial stiffness, and it is generally believed that chronic rather than short-term exercise is needed to modify this cross-linking. Functionally, increased sympathetic activity during MVPA may improve actions of vascular smooth muscle to stimulate vasodilatory properties and increase bioavailability of endothelial nitric oxide. MVPA also improves other cardiovascular risk factors, such as blood pressure, lipids and lipoproteins, and glucose-insulin metabolism, which in turn could indirectly improve arterial stiffness and vascular health. The relative importance of the direct or indirect action of increased MVPA and CRF on the vasculature for determining central arterial stiffness has not been determined.

Research regarding the influence of MVPA on arterial stiffness is limited, and more specifically, the type and intensity of activity necessary to produce an effect has not been well documented. Daily walking time has favorably shown to be inversely associated with cfPWV in an obese cohort, while non-structured MVPA has revealed weak associations, although available data is self-reported. Data relating CRF and arterial stiffness is present, revealing higher levels of CRF to be associated with 13-25% greater arterial compliance when compared to lower fitness levels. These associations have been observed in exercise interventions without
significant weight change. In addition, the relationship is observed in both men and women with varying levels of MVPA. Change in weight is known to influence cfPWV and it remains unclear if the contribution of CRF to improved arterial stiffness is additive or independent of changes in weight and abdominal fat. Furthermore, limitations of current literature on MVPA and arterial stiffness indicate that most studies use self-reported measures and are often cross-sectional or short-term. The proposed study will utilize objectively-measured MVPA to better answer the question of how MVPA influences arterial stiffness in overweight and obese adults in a SBWL.

1.2.3 Arterial Stiffness and Lifestyle Intervention

Carotid-femoral pulse wave velocity has been shown to improve after SBWL even with only modest weight reductions, but the independent contributions of changes in ‘fatness’ (overall and abdominal obesity) and ‘fitness’ (MVPA and CRF) are not well understood. The influence of overall obesity is unclear in middle-aged adults and abdominal obesity has not been studied in relation to cfPWV in long-term interventions. In addition, objectively-measured, non-structured MVPA in the context of arterial stiffness has only been studied in one short-term intervention, which warrants further study of objectively-measured MVPA in SBWL. Furthermore, the effects of CRF on cfPWV have not been assessed in the context of weight and abdominal fat loss over longer term SBWL. Further information on whether increases in MVPA and CRF provide additional benefit beyond weight loss during the initial phase of SBWL would be informative for planning interventions to improve arterial stiffness.
1.3 GAPS IN THE LITERATURE

Lifestyle interventions can produce a 10% weight loss if adherence to intervention components is successful. In addition to total weight loss and abdominal fat loss, increases in MVPA and improved CRF are often achieved. Changes in weight and abdominal fat are impactful for reducing arterial stiffness along with increased MVPA and CRF. Arterial stiffness has shown to be a valuable subclinical measurement for identifying early risk of future CVD. There is a need to study the effects of CRF and MVPA changes on cfPWV in long-term SBWL. More specifically, whether these associations exist beyond changes in weight and abdominal fat is not well understood. Further experimental evidence using high quality measurement methodology for arterial stiffness, abdominal obesity, MVPA, and CRF is needed to fill these gaps. The purpose of this study was to determine if 6-month changes in overall and abdominal obesity, CRF, and MVPA were related to 6-month changes in cfPWV in overweight and obese adults, and if the relationship between changes in CRF and MVPA with cfPWV occurred independently of changes in overall and abdominal obesity.
1.4 SPECIFIC AIMS

1) To examine the overall relationships between:

   a) 6-month changes in overall obesity (body weight, BMI, and % total body fat) and carotid-femoral pulse wave velocity (cfPWV) in overweight and obese adults enrolled in a standard behavioral weight loss intervention (SBWL).

   b) 6-month changes in abdominal obesity measured by dual-energy X-ray absorptiometry (DXA) and cfPWV in overweight and obese adults enrolled in a SBWL.

   c) 6-month changes in cardiorespiratory fitness (CRF) measured by a graded exercise test and cfPWV in overweight and obese adults enrolled in a SBWL.

   d) 6-month changes in objectively-measured physical activity (MVPA) and cfPWV in overweight and obese adults enrolled in a SBWL.

2) To determine if changes in CRF have an effect on cfPWV, independent of changes in overall obesity and abdominal fat.

3) To determine if changes in objectively-measured MVPA have an effect on cfPWV, independent of changes in overall obesity and abdominal fat.
1.5 HYPOTHESES

1) There will be significant associations between 6-month changes in overall obesity, abdominal fat, CRF and objectively-measured MVPA with cfPWV.
   a) There will be a significant positive association between 6-month changes in body weight, BMI, and % total body fat with cfPWV.
   b) There will be a significant positive association between 6-month changes in abdominal fat and cfPWV.
   c) There will be a significant inverse association between 6-month changes in CRF and cfPWV.
   d) There will be a significant inverse association between 6-month changes in objectively-measured MVPA and cfPWV.

2) Increases in CRF will be related to decreases in cfPWV, independent of changes in overall and abdominal obesity.

3) Increases in MVPA will be related to decreases in cfPWV, independent of changes in overall and abdominal obesity.
1.6 THEORETICAL RATIONALE

Figure 1 illustrates the theoretical framework by which a SBWL can elicit reductions in cfPWV. Such interventions have been shown to produce a 10% reduction in initial body weight as well as reductions in abdominal fat. Furthermore, interventions with an exercise component seek to improve MVPA levels and CRF.

Figure 1. Theoretical model for improving pulse wave velocity by standard behavioral weight loss intervention
1.7 SIGNIFICANCE

Carotid-femoral pulse wave velocity is a marker of aortic stiffness, is worsened in obese individuals and has been shown to be an independent predictor of cardiovascular health outcomes such as stroke, coronary heart disease and cardiovascular mortality.\textsuperscript{14,74} Weight loss is a primary recommendation for cardiovascular health in obese populations. There is evidence that weight loss improves cfPWV independently from other obesity-related factors and that reductions in abdominal fat appear to confer additional benefit.\textsuperscript{43,44} Furthermore, modest improvements in CRF and self-reported MVPA have been shown to elicit reduced cfPWV.\textsuperscript{45,56,64,69,75} However, in the context of long-term SBWL that reduces overall obesity and abdominal fat, the impact of increased CRF and objectively-measured MVPA on cfPWV is not well understood.

The purpose of this study was to determine if 6-month changes in overall and abdominal obesity, CRF, and MVPA were related to 6-month changes in cfPWV in overweight and obese adults, and if the relationship between changes in CRF and MVPA with cfPWV occurred independently of changes in overall and abdominal obesity. Further knowledge of the independent and combined effects of these SBWL intervention components could be beneficial for designing interventions that aim to reduce cfPWV and slow the progression of chronic arterial disease.
2.0 REVIEW OF LITERATURE

2.1 INTRODUCTION

Cardiovascular disease describes any condition involving the heart, blood vessels, or both and is the leading cause of morbidity and mortality in the United States.\textsuperscript{76} Physiological parameters, such as blood pressure, lipids, and fasting glucose are traditionally assessed to evaluate the risk of future cardiovascular outcomes such as heart attack, stroke, and heart failure. Vascular conditions, such as arterial stiffness and endothelial dysfunction, have also been established as independent predictors for cardiovascular risk.\textsuperscript{11,77} These are thought to be intermediate endpoints for cardiovascular disease,\textsuperscript{4} or early, subclinical manifestations of adverse future health outcomes such as hypertension, stroke, and ventricular failure.\textsuperscript{5}

Lifestyle interventions that include calorie restriction, increased MVPA or both are capable of improving arterial stiffness.\textsuperscript{42-44,72,73} However, the independent effects of changes in MVPA and CRF on arterial stiffness, independent of change in body composition are not well understood. Understanding the additive benefits of MVPA and CRF in a weight loss intervention would be useful for designing effective programs to reduce arterial stiffness.
2.2 ARTERIAL STIFFNESS

2.2.1 Pathophysiology

The pathophysiology of arterial stiffness can occur throughout the entire vasculature, but increased stiffness of central arteries (i.e. aortic stiffness) is more consistently associated with cardiovascular outcomes when compared to peripheral artery stiffness. Aortic stiffness adds predictive value to cardiovascular risk in addition to established cardiovascular risk factors, atherosclerosis, and pulse pressure. Thus, the following review will primarily discuss aortic or central stiffness.

Within the arterial system, collagen and elastin serve as the primary molecular structures and undergo a continuous process of production and degradation. An imbalance in this system, typically from high arterial pressure and inflammatory factors, leads to a favored production of collagen and diminished elastin. Arterial stiffness, a type of arteriosclerosis, is a progressive ‘hardening’ or ‘stiffening’ of the arterial wall that results from this shift in structure, primarily within the intima and media. Healthy arteries contain a greater amount of elastin fibers than collagen to facilitate blood flow from larger arteries to smaller arterioles and capillaries. A large ratio of elastin fibers is especially important in the aorta and its main branches in order to cushion blood flow to distal arteries. Arterial stiffness develops when elastin fibers are gradually replaced with more rigid collagen fibers and this change is accompanied by increased fiber cross-linking, glycalated end products, growth factors and hypertrophy of vascular smooth muscle cells to yield a stiffer artery. Structural changes occur as part of the aging process and are exacerbated by shear stress forces, neurohormones, lipids, glucose regulation and other extrinsic forces.
It has been established that arterial stiffness is more related to conditions of vascular overload (e.g. left ventricular hypertrophy and chronic heart failure) than acute, ischemic events (e.g. myocardial infarction), and these conditions appear to be, in part, mediated by the onset of hypertension.\textsuperscript{71} This stiffening process is not to be confused with atherosclerosis, which is a consequence of arterial plaque build-up and manifests by separate pathways.\textsuperscript{85} However, the presence of atherosclerosis in the blood vessels can promote an inflammatory environment which is thought to exacerbate the development of arterial stiffness.\textsuperscript{86,87}

\textbf{2.2.2 Assessment Methods of Arterial Stiffness}

Carotid-femoral pulse wave velocity is considered the gold standard for arterial stiffness assessment and the measurement has been well validated against invasive, direct measures of pressure, blood flow and vessel diameter changes.\textsuperscript{9} The theoretical model is based on the Moens-Korteweg equation, which is described as:

\[ \text{PWV} = \sqrt{\frac{Eh}{2rp}} \]

where $E=$slope of the stress-strain relationship for a vessel, $h/2r=$wall diameter, and $p$ is the density of fluid.\textsuperscript{17} In clinical assessment, cfPWV captures the transit time of a pressure waveform that travels through the arterial branches following an ejection of blood from the heart. Following ventricular contraction, a pressure wave is generated and is propagated forward during the systolic phase throughout the arterial tree. In normal elastic arteries, the wave is reflected back to the heart at the initiation of diastole. As arteries become stiffer, the pulse wave returns to the heart earlier, during late systole. This faster response heightens sympathetic tone, which amplifies systolic blood pressure and increases cardiac workload. Carotid-femoral pulse wave
velocity is how fast the pulse wave travels over time, often measured in meters per second (m/s). A slower velocity is indicative of a healthier and more elastic artery, while a faster velocity reflects a stiff artery.

Carotid-femoral pulse wave velocity is well established as an intermediate endpoint for cardiovascular outcomes. Longitudinal studies have found associations between higher cfPWV and cardiovascular outcomes, specifically myocardial infarction, stroke, ventricular heart failure, cardiovascular mortality, and all-cause mortality. The most recent meta-analysis from 2010 found that a one meter per second higher cfPWV was associated with a 15% increase in the risks of cardiovascular events, cardiovascular mortality and all-mortality. A one standard deviation higher cfPWV was associated with a 47% greater risk of these outcomes. After compiling 17 longitudinal studies (N=15,877), the authors showed that in a 7.7 year period, those with higher cfPWV (mean ≥11.3 m/s) had twice the risk for an event compared to those with lower cfPWV (≤11.3 m/s). These risks persisted with adjustment for age, gender and other risk factors, in hypertensives and non-hypertensives and even after adjustment for Framingham Risk Score.

Carotid-femoral pulse wave velocity is commonly assessed in epidemiological research by applanation tonometry. This technique captures pulse waveforms at the superficial carotid and femoral arterial points via piezoelectric (pressure) sensors, which are placed on the skin and able to calculate the difference in transit time between the 2 sites. A foot-to-foot methodology is used, in which the beginning (i.e. “foot”) of the waveforms serves as the reference point for transit time calculation. Carotid-femoral pulse wave velocity measured by applanation tonometry consistently associates with cardiovascular outcomes, independent of other risk factors. It should be noted that the estimation of aortic length required for cfPWV
may be a limitation in an obese population, as a larger abdominal area could influence the validity of the measure.\textsuperscript{98}

Magnetic resonance imaging (MRI) is an alternative method for cfPWV measurement, often used in studies with pathological interest.\textsuperscript{27,99} In an obese population, cfPWV measured by MRI is moderately correlated with values from various tonometry devices (Pulse Pen: $r=0.47$, $p=0.005$, Complior: $r=0.43$, $p=0.01$).\textsuperscript{98} MRI directly and noninvasively measures aortic length using 2 or 3-dimensional images with excellent spatial and temporal resolution.\textsuperscript{98,100} The technique is costly and therefore, not typically used in epidemiological research.

Other methods to assess arterial stiffness include ultrasound, pulse waveform analysis, pulse pressure, and oscillometric blood pressure. Pulse pressure, the difference between systolic and diastolic blood pressures, is measured by standard sphygmomanometry and provides a crude estimate of arterial stiffness.\textsuperscript{8} Pulse pressure provides a crude estimate of arterial stiffness, and above normal values may indicate arterial stiffness.\textsuperscript{101} Although an easy measurement, peripheral pressures are only an estimation of central pressure and may not be an accurate reflection of systemic arterial stiffness.\textsuperscript{102} Pulse wave analysis, like cfPWV, is assessed by applanation tonometry and records pressures at peripheral artery sites (radial or carotid) and derives a corresponding central waveform. This technique takes into account the forward pressure wave as well as the portion reflected back toward the heart, which is used to derive augmentation index. Ultrasound is a traditional Doppler method on which older literature on cfPWV is based. Ultrasound is a more expensive alternative and can assess arterial stiffness by assessment of elasticity properties, directly determining how changes in pressure influence changes in volume and diameter. Images of the vessel wall are captured and must be of sufficient clarity for valid interpretation. This technique requires more extensive operator
training and therefore is utilized more often in pathophysiological and pharmacological studies, rather than epidemiological research.\(^8\)

### 2.2.3 Risk Factors

Aging is the largest contribution to vascular stiffness and these effects are independent of other risk factors and conditions.\(^{82,103,104}\) Arterial stiffness progresses approximately 10-15\% per decade (1\% per year)\(^{105}\) and promotes age-related increases in pulse pressure and systolic hypertension.\(^5\) Arterial stiffness appears to increase more rapidly in women than men starting around the age of 45, likely due to hormonal shifts.\(^{106}\) Along with the natural progression of structural changes that occur with age, the presence of other risk factors further accelerates the process.\(^{107}\)

Blood pressure is consistently correlated with measures of arterial stiffness, more specifically the incidence of hypertension.\(^{15,108-110}\) To an extent, the magnitude of stress and strain on the vessel wall regulates pressure wave conductance in the aorta and increases arterial stiffness.\(^{111}\) Longitudinal studies have since suggested a temporal relationship that stiffness precedes hypertension.\(^{14,15,112}\) With increasing stiffness, extra pressure or “loading” is placed on the left ventricle and is accompanied by augmented systolic pressure and a reduction in early diastolic pressure.\(^{113}\) In the Atherosclerosis Risk in Communities study, each standard deviation lower in arterial elasticity increased the risk of incident hypertension by 15\% in initially normotensive middle-aged adults.\(^{71}\) Similarly, from a cohort of 1759 in the Framingham Study, arterial stiffness predicted future systolic blood pressure, pulse pressure and incident hypertension.\(^{15}\)
Other contributing risk factors to arterial stiffness are less established. Conditions of metabolic syndrome, diabetes mellitus and dyslipidemia have shown associations with arterial stiffness, but are currently not well established and require further investigation. The inclusion of lifestyle factors such as smoking in arterial stiffness literature is present, although to a lesser degree. Cigarette smoking has been shown to increase stiffness measures in normotensive and hypertensive individuals acutely. Long-term effects of smoking interestingly revealed no differences between smokers and non-smokers in at least 2 studies. Obesity and MVPA have also been studied and will be discussed in the following sections.

2.3 OBESITY

2.3.1 Abdominal Obesity

Obesity remains a primary health concern in the United States and has been well established as a risk factor for cardiovascular morbidity and mortality. The 2011 National Health and Nutrition Examination Survey (NHANES) reports 68.8% of Americans to be overweight and 34.9% to be obese. Obesity is a chronic metabolic disorder that is characterized as excess energy stored as adipose tissue and is accompanied by increases in blood volume, cardiac output, and cardiac workload. To maintain the hemodynamic demands of this excess mass, the vasculature must overcompensate and such alterations often have chronic implications, such as hypertension and left ventricular hypertrophy. A positive association between body weight and left ventricular mass has been demonstrated and the hypertrophic effect leads to further
dysfunction of the myocardium. The remodeling of vascular structure and hemodynamic function as a result of increased blood volume and cardiac afterload is also accompanied by exaggerated sympathetic activity, all of which may explain the impact of obesity on arterial stiffness. Chronic stress on the arterial wall prolongs and augments the systolic phase, reduces vessel compliance and elasticity and is thought to eventually lead to arterial stiffness. These changes increase risks of ventricular hypertrophy, heart failure and mortality. The risk of heart failure increases across higher BMI categories and, compared with normal weight, obese individuals have twice the risk for heart failure. Furthermore, with each 5 kg/m² higher BMI, cardiovascular mortality and overall mortality risks increase by 40% and 30%, respectively.

Other obesity-related mechanisms contribute to changes in cfPWV. These include excessive lipids, proinflammatory factors and insulin resistance, although specific pathways are still under investigation. Adipose tissue is considered an inflammatory organ and releases proinflammatory cytokines or adipokines in obese conditions. With higher obesity, adipocyte cells hypertrophy and secrete additional adipokines that exacerbate inflammatory damage to the vasculature. As traditional adipose tissue exceeds normal storage capacity and adipose cells accumulate in other tissues, normal metabolic and hemodynamic function is disrupted, often presented as sympathetic dominance and insulin resistance. In the Framingham Study, an estimated 65% to 78% of cases of hypertension could be attributed to obesity, likely due to greater oxidative stress and proinflammatory adipokines. The conditions of obesity and arterial stiffening become a chronic, cyclical pattern that can lead to serious clinical outcomes.

Higher cfPWV is observed in obese individuals when compared to lean counterparts even after correction for traditional risk factors such as cholesterol, blood pressure, glucose and insulin. When matched for age, cfPWV is approximately 0.5 meters per second higher in
obese individuals compared to non-obese.\textsuperscript{25} Toto-Moukouo et al. were the first to evaluate arterial stiffness in the context of obesity and found a positive correlation \((r=0.85, p<0.001)\) between degree of obesity and cfPWV.\textsuperscript{124} Generally, the relationship between obesity and aortic stiffness persists between genders and across age groups, but studies have produced mixed results.\textsuperscript{37-40} Zebekakis et al. studied 1306 subjects and found that BMI was a significant predictor of cfPWV in women but not men and correlations were weak within groups after age stratification.\textsuperscript{125} Wildman et al. found obesity measurements (body weight, BMI, waist circumference, and waist-hip ratio) to be strong predictors of cfPWV in both younger (age 20-40) and older adults (age 41-70). In older adults only, the overweight individuals (BMI between 25.0 – 29.9 kg/m\(^2\)) demonstrated higher cfPWV values than normal weight counterparts (BMI <25.0 kg/m\(^2\)) and cfPWV levels approached those of the obese individuals (BMI ≥30 kg/m\(^2\)).\textsuperscript{25} Other studies report an age-dependent effect where higher obesity level is associated with lower cfPWV in young adults, but then becomes associated with higher cfPWV after age 50.\textsuperscript{38,39} These results may suggest the impact of obesity on stiffening to be a cumulative effect that manifests later in life. Since age is the most established risk factor for cfPWV, continued study of various age groups in the context of obesity is important for improved risk evaluation. Effects of overall obesity on cfPWV remain somewhat inconclusive in middle-aged adults, as some longitudinal results report weak associations between obesity and cfPWV in this population.\textsuperscript{37-39} Since these individuals are at a higher risk for conditions such as hypertension compared to younger adults, further study of this population could be of value for primary prevention of cardiovascular outcomes.\textsuperscript{126,127}
2.3.2 Weight Loss

Weight loss is a recommendation for improving obesity-related arterial stiffness. Decreases in blood volume and cardiac output with weight reduction are primary driving forces in mechanically altering the vasculature.¹²⁸ Neurohumoral and sympathovagal adaptations acutely improve arterial stiffness after weight loss⁴³ while structural improvements of the artery, such as protein cross-linking, are related more to the chronic impact of weight loss.⁴⁴ Nitric oxide bioavailability and renin-angiotensin-aldosterone activity may also influence arterial stiffness²⁷ and improvements in insulin and metabolic factors are also believed to contribute to stiffness reductions after weight loss.²⁷,¹²⁹

Weight change has been shown to influence arterial stiffness in longitudinal, observational studies; weight gain has been linked to arterial stiffness progression, while weight loss is associated with regression and reduced CVD risk.⁴¹,⁵⁴ Wildman et al. observed the greatest increases in cfPWV (4% per year progression) in those who gained the most weight (≥4.5 kg per year) over a 2 year period.⁴¹ Furthermore, baseline obesity measures were independent of changes in obesity measures, suggesting that the effects of weight gain on the vasculature occur regardless of initial body weight. The reverse phenomenon, regression of arterial stiffness, was observed with weight loss in this study and is promising for future weight loss interventions.

Standard behavioral weight loss interventions are typically comprised of calorie restriction, increased MVPA and behavior therapy, and are recommended by the National Institutes of Health for successful weight loss.⁷³ Experimental studies with SBWL that produce modest weight loss (5-10% of baseline body weight) have shown improvements in arterial stiffness measures independent of other risk factors.⁴²-⁴⁵ Some experimental evidence reveals
that weight change is associated with change in cfPWV, but baseline relationships are not always assessed and data is rather limited. Rider et al. found cfPWV to be 14% higher in severely obese individuals compared to lean, and large weight loss (50% excess weight) to improve cfPWV by 14%, essentially matching normal weight controls. This improvement was associated with body mass index reduction ($r=0.39$, $p=0.05$), but not with any metabolic, hormonal or blood pressure changes. Cooper et al. found baseline BMI to be associated with cfPWV independent of other obesity and hemodynamic factors in young adults and a 5% cfPWV reduction with modest average weight loss of 7%.

Goldberg et al. conducted a 6-month nutrition and exercise intervention and divided subjects into three groups based on post-intervention weight loss (>10%, 5-10%, <5%). They found that improvements in arterial stiffness were significantly less in those with <5% weight loss ($p<0.0001$), and there were no significant differences when comparing the other 2 groups. Other weight loss intervention studies achieving similar modest weight loss show reduced arterial stiffness in healthy obese, type 2 diabetics and hypertensives.

### 2.4 MODERATE-VIGOROUS PHYSICAL ACTIVITY AND CARDIORESPIRATORY FITNESS

Low levels of CRF and a lack of MVPA are well established risk factors for cardiovascular disease. Moderate-vigorous physical activity includes: 1) exercise that is planned, structured activity and 2) non-exercise activity that may include occupational, recreational or sport activity, although research of MVPA is often limited to a specific component of MVPA (e.g. exercise or non-exercise activity). Though related, CRF is distinct in that it is the physiological
response of body systems to supply oxygen during MVPA.\textsuperscript{61} It has been shown that higher CRF is associated with 13-25\% higher arterial compliance when compared to lower fitness levels.\textsuperscript{45,69} Incidence of cardiovascular disease is higher in individuals with lower CRF and lower MVPA levels,\textsuperscript{58,59} making these important factors to target in future intervention studies.

The mechanisms through which CRF or MVPA improves arterial stiffness have been explained as both structural and functional adaptations of the vessels.\textsuperscript{63,64} Increased cross-linking of collagen and elastin fibers is the primary contributor to arterial stiffness, and it is generally believed that chronic rather than short-term exercise is needed to modify this cross-linking.\textsuperscript{64} Functionally, increased sympathetic activity during MVPA may improve actions of vascular smooth muscle to stimulate vasodilatory properties and endothelial nitric oxide.\textsuperscript{56,65} Weight loss improves stiffness by reducing blood volume and inflammatory factors, but the effects of exercise may be more closely related to better vasodilatory responses that induce acute adaptations in the vasculature.\textsuperscript{56} Moderate-vigorous physical activity also improves other CVD risk factors, such as BP, lipids and lipoproteins, and glucose-insulin metabolism, which in turn could indirectly improve arterial stiffness and vascular health.\textsuperscript{66,67} The relative importance of direct or indirect action of increased MVPA and CRF on the vasculature and central arterial stiffness has not been determined.

Associations between arterial stiffness and CRF have been well-established, while measures of MVPA, mostly self-reported, have yielded somewhat inconsistent results. Assessment of CRF and MVPA status in relation to arterial stiffness has largely focused on determining if improved aerobic capacity can blunt age-related stiffness. The Baltimore Longitudinal Study of Aging studied older men (ages 54-75) and found arterial stiffness to be 26\% lower in endurance-trained men compared to their sedentary peers without significant
differences in resting blood pressure.\textsuperscript{70} Tanaka et al. studied middle-aged and older men (age 38-77) who were either recreationally active (moderate exercise at least 3 days per week) or highly trained (vigorous exercise or competing in endurance events). In the highly trained group, exercise blunted the age-related increases in arterial stiffness by nearly 50\% and positive trends were observed in the recreationally active group.\textsuperscript{64} In a separate study, Tanaka et al. compared sedentary and active pre- and post-menopausal women and observed a similar result. The post-menopausal sedentary women showed arterial stiffness to be 16.5\% higher than the pre-menopausal sedentary, while no significant differences were seen between the active groups.\textsuperscript{6} These studies suggest that greater levels of exercise and CRF may be protective against age-related declines in arterial stiffness.

The addition of work, recreational and leisure activities to studies of cardiovascular health has been important for filling the gap between sedentary behavior and structured exercise. The Atherosclerosis Risk in Communities sought to determine if arterial health was associated with work, leisure, or sport MVPA at varying intensities in adults aged 45-64. Weak associations were found only for self-reported work activity and vigorous sport activity in the overweight cohort before and after adjustment for blood pressure and other covariates.\textsuperscript{67} Even with a large sample size (N=10,644), these results could not support an association between leisure time MVPA (typically less vigorous activities) and improved arterial health, potentially limited by self-reported methods. Kupari et al. also utilized MVPA questionnaires and found higher levels of self-reported MVPA to be associated with decreased arterial compliance in non-obese subjects.\textsuperscript{132} These qualitatively different results are inconsistent with other related studies, possibly explained by self-report differences in measurement techniques or the intensity of activity. General limitations of the MVPA and arterial stiffness literature are that most studies
use self-reported measures and are often cross-sectional or short-term and this could contribute to the weak associations observed. In addition, whether increasing CRF has an independent effect on arterial stiffness in the context of weight loss remains unclear. The proposed study will utilize objectively-measured MVPA to better answer the question of how MVPA influences arterial stiffness within a SBWL.

Although some inconsistencies remain in the literature, general recommendations encourage increases in structured, aerobic exercise and MVPA for the prevention and treatment of arterial stiffness. The Activity Counseling Trial increased walking over 24 months in overweight/obese subjects and found self-reported walking to be inversely associated with cfPWV. In the same study, an independent association was found between CRF and cfPWV and this was accompanied by no significant weight loss. Collier et al. found just 4 weeks of aerobic training to improve cfPWV by 9.5%, without changes in blood pressure or body weight. These studies were isolated exercise interventions and did not include a dietary weight loss component. With the addition of weight management, the Exercise and Nutrition Interventions for Cardiovascular Health study achieved a 19% improvement in CRF in hypertensive individuals which was accompanied by significant blood pressure reductions and a 9% weight loss. In addition, cfPWV significantly improved and the authors hypothesized that increased CRF contributed to the reduction in arterial stiffness, but did not report statistical analysis to confirm this. In a pair-matched weight loss intervention, Balkestein et al. found the addition of aerobic exercise to an energy-restricted diet did not further improve arterial compliance. It should be noted that exercise was performed at 40% maximal capacity for 3 months and may have not been a sufficient stimulus to observe vascular change. More recently, a non-randomized 7-week trial in morbidly obese individuals compared a low calorie
diet (900 kilocalories/day) to a lifestyle intervention, comprised of calorie restriction (1000 kilocalories/day) and aerobic exercise 3 days per week. After adjustment for confounders, the lifestyle intervention group reduced cfPWV by 0.6 m/s ($p=0.001$) compared to 0.1 m/s ($p=0.064$) reduction in the low calorie only group. In addition, loss of fat mass was not significantly different between groups, but loss of muscle mass was larger in the low calorie group (0.8 kg, $p≤0.001$).

### 2.5 ARTERIAL STIFFNESS AND LIFESTYLE INTERVENTION

The research supporting that change in MVPA and CRF in lifestyle interventions has an effect on the change in cfPWV is limited. Currently, there is a need for intervention studies to assess the combined effects of these components on arterial stiffness using objective methods. In the context of weight management and body fat assessment, further information could be gained for designing lifestyle interventions for overweight and obese adults, targeting subclinical cardiovascular outcomes. Lifestyle interventions to increase structured exercise have produced favorable results, with a recent 7-week trial indicating aerobic exercise combined with calorie greater improvements in cfPWV in a lifestyle intervention group (calorie restriction + exercise) compared to a low calorie diet alone in obese individuals.\textsuperscript{134} The influence of overall obesity is unclear in middle-aged adults and abdominal obesity has not been studied in relation to cfPWV in interventions longer than 3 months. In the same study, objectively-measured MVPA was evaluated, but MVPA levels were not significantly increased, leaving conclusions regarding the effect of MVPA on cfPWV unclear.\textsuperscript{44} Additionally, the effects of CRF on cfPWV have not been assessed in the context of weight and abdominal fat loss over longer-term SBWL (≥3 months).
2.6 CLINICAL SIGNIFICANCE

The Framingham Heart Study, Atherosclerosis Risk in Communities and other longitudinal studies have observed temporal patterns of arterial stiffness preceding the onset of hypertension. Aortic stiffness causes premature return of the reflected wave in the systolic phase of contraction, which occurs during diastole in healthy arteries. This manifestation raises systolic blood pressure, chronically increases afterload on the heart and leads to conditions of cardiac hypertrophy and failure. Other biomarkers (such as lipids, CRP and glucose) give little information on their cumulative effect until an endpoint (e.g. myocardial infarction). Study of arterial stiffness may give insight into preventative measures of delaying outcomes and improving quality of life since stiffness is detectible before overt CVD symptoms.

2.7 GAPS IN THE LITERATURE

Standard behavioral weight loss interventions often target decreased dietary intake and increased MVPA and can produce a ≥10% weight loss if adherence to lifestyle approaches is successful. In addition to total weight loss and abdominal fat loss, increases in MVPA and improved CRF are often achieved. Changes in weight and abdominal fat are impactful for reducing arterial stiffness, which is a subclinical measure of cardiovascular disease and a valuable prognostic tool for risk of cardiovascular outcomes. Without caloric restriction, increased MVPA and CRF improve aortic stiffness, but there is limited objective data measuring MVPA. Furthermore, evidence examining the effects of CRF and MVPA changes on cfPWV in long-term SBWL is lacking. More specifically, whether these associations exist
beyond changes in weight and abdominal fat is unknown. To investigate these questions, larger, longer (>3 months) studies using high quality measurement methodology for arterial stiffness (cfPWV by tonometry), abdominal obesity (DXA), physical activity (objective), and CRF (graded exercise test) are needed. We propose to address these gaps by studying the overall and independent relationships between changes in overall and abdominal obesity with change in cfPWV among overweight and obese adults over a 6-month SBWL.
3.0 METHODS

The current study is ancillary to a parent study, an ongoing randomized clinical trial to primarily assess changes in left ventricular mass by cardiac MRI in response to a 12-month SBWL with various MVPA prescriptions. The program is modeled after well-established theoretical models of lifestyle behaviors and previously successful interventions conducted by the investigators. A summary of the parent study procedures is outlined below.

3.1 INTERVENTION ARMS

The current study will not assess or analyze any outcomes by intervention arm; such comparisons will occur upon completion of the parent study in early 2016. A brief description of the interventions is outlined below for purposes of providing an overview of the parent study.

DIET ONLY: Subjects received intervention including an energy intake prescription of 1200-1800 kilocalories per day (kcal/d) and 20-30% dietary fat intake based on initial body weight. A physical activity prescription was not provided to this group.

DIET + MODERATE PHYSICAL ACTIVITY: Subjects received intervention including an energy intake prescription identical to the DIET group. In addition, subjects were prescribed 150 minutes per week of moderate intensity exercise (55-70% age-predicted maximal heart rate), which was progressed in a gradual manner.
**DIET + HIGH PHYSICAL ACTIVITY:** Subjects in all arms received an intervention including an energy intake prescription identical to the DIET group. In addition, subjects were prescribed 250 minutes per week of moderate intensity exercise (55-70% maximal heart rate), which was progressed in a gradual manner.

**Months 1-6:** Subjects in all groups reported weekly to the University of Pittsburgh Physical Activity and Weight Management Research Center (PAWMRC) for one-hour group sessions where staff members delivered information pertaining to the assigned behavioral weight loss intervention (nutrition, exercise, and other lifestyle factors). Body weight was tracked and self-monitoring paper diaries (reporting foods, calories, fat grams, exercise) were turned in to intervention staff for review and feedback.

**Months 7-12:** All subjects reported bi-weekly to the PAWMRC for the group sessions outlined above, and received a phone call from the intervention staff on weeks without a group session. Phone calls addressed strategies for meeting goals and any other specific concerns.

### 3.2 RECRUITMENT AND SCREENING PROCEDURES

Subjects were recruited through postcard mailings, newspaper and radio advertisements. Letters were also mailed to potentially eligible individuals registered in the Obesity and Nutrition Research Center Clinical Registry. Interested individuals were instructed to call the PAWMRC. Trained staff and graduate students provided a description of this study and, after verbal consent from the individual, followed phone screening procedures to determine initial eligibility. Questions including demographic information, medical history, and general physical health were asked. Those found to be eligible based on phone screening were asked to provide contact
information and later invited to an orientation session conducted by the Principle Investigator. Detailed study procedures were given and informed consent was provided for further participation. All study procedures were approved by the Institutional Review Board at the University of Pittsburgh.

3.3 SUBJECTS

Apparently healthy men and women between the ages of 18-55 were recruited for the parent study. Subjects were overweight, Class I, and Class II obese according to BMI classification (25.0-39.9 kg/m²), and were sedentary defined as <60 minutes per week of moderate-intensity exercise over the last 3 months. Exclusionary criteria for the parent study were as follows:

1) Diagnosis of heart disease, angina, uncontrolled arrhythmia, or previous cardiovascular event, coronary artery bypass grafting or angioplasty.

2) Resting systolic blood pressure ≥160 mmHg or resting diastolic blood pressure ≥100 mmHg or currently taking hypertensive medications, because hypertensive individuals planning to engage in vigorous activity should be medically supervised.\textsuperscript{135}

3) Conditions affecting heart rate, blood pressure, or metabolism to include diabetes mellitus, thyroid, cancer (current treatment or within the previous 5 years), chronic renal insufficiency or chronic liver disease.

4) Current treatment for an eating disorder.

5) Current diagnosis or treatment for psychological disorders, or taking psychotropic medications that could influence body weight or interfere with the intervention and outcome measures.
6) Women who are pregnant, breastfeeding, or have been pregnant in the previous 6 months.

7) Current participation in a weight loss program, ≥5% weight loss in the previous 3 months or taking medications for weight reduction which would confound the parent study intervention and outcome measures.

8) Any physical limitation that would prevent the ability to walk for exercise, which was a component of some of the intervention arms.

3.4 ASSESSMENT PROCEDURES

Subjects reported to the PAWMRC for 2 assessment visits at 0 and 6 months. All described assessment measures were performed at both time points. Prior to arrival, assessment instructions included: 1) no food or beverages (excluding water) 12 hours prior, 2) no alcoholic beverages 24 hours prior, 3) no vigorous activity 24 hours prior, 4) no smoking 24 hours prior, and 5) lightweight, comfortable clothing and shoes for treadmill walking. Deviation from this protocol could compromise cardiovascular measurements, which were of particular interest in the current study. Subjects reporting noncompliance to these instructions were asked to repeat the assessment on another day.

3.4.1 Body Height, Weight, and Body Mass Index

Height was measured using a wall-mounted stadiometer (Perspective Enterprises; Portage, MI) to the nearest 0.1 cm. Subjects were dressed in a hospital gown and asked to remove shoes prior
to measurement. Two height measurements were taken and, if there was ≥0.5 cm difference, a third was taken. Body weight was measured to the nearest 0.1 kg using a digital scale (Tanita Corporation; Arlington Heights, IL). Two weight measurements were taken and, if there was ≥0.2 kg difference, a third was taken. Body mass index was calculated as kg/m².

### 3.4.2 Body Composition

Total body composition (% body fat) was assessed using a GE Lunar dual-energy X-ray absorptiometer (iDXA) (GE Healthcare; Madison, WI) with participants in a hospital gown and all metal and jewelry removed. Female subjects completed a urine pregnancy test prior to examination and were excluded if testing positive. This is a safety precaution due to a small amount of radiation exposure from the absorptiometer. Abdominal obesity, specifically abdominal fat (kg) and (%) was also assessed from DXA scans using enCORE software version 14.10.022. The abdominal region was defined by using the diaphragm as the cephalad boundary and the iliac crest as the caudal boundary.

### 3.4.3 Circumference Measurements

Waist circumference measurements were taken to the nearest 0.1 cm using a Gulick tape measure with participants in a hospital gown. Two measurements were taken and, if there was ≥1.0 cm difference, a third was taken. Waist circumference is often utilized in weight loss research as an acceptable surrogate measure for abdominal obesity.¹³⁶
3.4.4  **Seated Resting Heart Rate and Blood Pressure**

Resting heart rate and blood pressure were obtained using a Dinamap V100 (GE Healthcare) with subjects in a seated position. After a 5-minute rest period, 2 measurements were taken with a one-minute rest period given between measurements. A third measurement was taken if systolic blood pressures differed by ≥10 mmHg or diastolic blood pressures by ≥6 mmHg.

3.4.5  **Cardiorespiratory Fitness (CRF)**

Cardiorespiratory fitness was assessed using a sub-maximal graded exercise test. Treadmill speed was held constant at 3.0 mph and treadmill grade was initially set at 0% and increased by 1% every minute. Heart rate and 12-lead ECG were continuously monitored by an American College of Sports Medicine Clinical Exercise Specialist. Oxygen consumption was measured by spirometry using a CareFusion Vmax Encore metabolic cart (San Diego, CA). Blood pressure and Rating of Perceived Exertion were assessed every minute to ensure appropriate physiological responses to exercise. The test was terminated if one of the following was observed: 1) achievement of 85% of maximal heart rate (220-age), 2) ECG or BP abnormality, 3) request of subject due to extreme fatigue, dizziness, nausea, etc., or 4) technical failure. Safety to exercise and participate in the parent study intervention was determined by completion of the exercise test and physician review of the ECG. Subjects not achieving 85% of maximal heart rate were excluded from fitness analyses.
3.4.6 Moderate-Vigorous Physical Activity (MVPA)

Moderate-vigorous physical activity was objectively assessed using the multisensor SenseWear Pro Armband (Body Media, Inc.; Pittsburgh, PA). The multi-sensor armband includes a 3-axis accelerometer to measure motion and steps and uses Galvanic skin response to measure energy expenditure. The armband provides minute-by-minute data in metabolic equivalents (METS) using proprietary algorithms and has been well validated in previous exercise and weight loss studies.\textsuperscript{138,139} Subjects were instructed to wear the armband during all waking hours (except for showering, bathing, swimming) for a period of one week. A minimum of 4 days with \( \geq 10 \) hours of armband data was required for analysis of MVPA.

For this study, MVPA was defined as the average daily total of minutes at \( \geq 3 \) METS, consistent with the American College of Sports Medicine guidelines.\textsuperscript{62} Definitions of MVPA evaluated included total weekly minutes of MVPA accumulated in one-minute bouts, total weekly minutes of MVPA accumulated in 10-minute bouts, total weekly MET-minutes of MVPA accumulated in one-minute bouts and total weekly MET-minutes of MVPA accumulated in 10-minute bouts.

3.4.7 Carotid-Femoral Pulse Wave Velocity (cfPWV)

Carotid-femoral pulse wave velocity is the gold standard method to assess central arterial stiffness\textsuperscript{93,140} and is the primary outcome of the current study. Carotid-femoral pulse wave velocity testing was performed using the Complior Analyse (Alam Medical; Vincennes, France) by a trained technician, certified by the Ultrasound Research Lab at the University of Pittsburgh.
The procedure is non-invasive and presents no serious risk to the participant. All measurements were performed on the right side of the body in a hospital gown.

### 3.4.7.1 Pre-assessment

Carotid-femoral pulse wave velocity assessment was performed between the hours of 7:00 A.M and 10:30 A.M. prior to cardiorespiratory fitness testing.

Prior to assessment subjects were asked the following questions:

1) “Have you eaten in the past 12 hours?” If responding “yes,” the time is noted.
2) “Have you had coffee, tea, or other caffeine in the past 12 hours?” If responding “yes,” the time is noted.
3) “Have you exercised in the past 24 hours?” If responding “yes,” the time is noted.
4) “Have you smoked in the past 24 hours?” If responding “yes,” the time is noted.

Refrain from food, caffeine, exercise and smoking are standard procedures for cfPWV analysis. A response of “yes” to any question was not exclusionary, but was noted for quality control purposes.

Subjects were instructed to lay supine for a 10-minute rest period, during which automated BP was taken using a Dinamap cuff. Aortic distance was estimated by subtracting distances from the carotid pulse to sternal notch and sternal notch to femoral pulse, which is widely used in PWV research. To minimize measurement error, the tape measure was held taut above the contours of the body.
3.4.7.2 Assessment

Piezoelectric sensors were placed at the carotid and femoral arteries where the pulses were most strongly felt. Sensors were held in place by the technician until 10 valid pulses, appearing as waveforms on the computer, were counted with low error (ideally ≤5%, but ≤10% if measurement is difficult). Once these valid waveforms were obtained, a second technician presses a “valid” button on the computer to capture the waveforms. The capturing of 10 valid waveforms counts as one scan and the procedure was repeated until 3 scans were obtained. cfPWV was calculated as the aortic distance divided by the time differential from the foot of a carotid waveform to the foot of the corresponding femoral waveform (e.g. foot-foot method) in meters per second (m/s). The cfPWV from the 10 individual waveforms are averaged by the computer for a final cfPWV. Ideally, 3 scans are obtained for each subject.

3.4.7.3 Data Processing and Quality Control

All scans were given a quality score based on the error within and across the 3 scans and poor quality scans were excluded or evaluated for influence. Procedures for the grading of quality are described below.

For each scan, the Compliors software calculated a mean cfPWV from 10 waveforms and a standard deviation of the cfPWV across the 10 waveforms. Ideally, the standard deviation was <5% of the mean cfPWV, but up to ≤10% was tolerated for difficult scans. Scans with >10% error were discarded.

After excluding scans with high variability, the maximum absolute difference in mean cfPWV across remaining scans was calculated. Ideally, this number was <0.5 m/s, but up to <1.0 m/s was tolerated. If the maximum difference was >1.0 m/s across scans within an individual at a given assessment period, the original waveforms were consulted to determine
which set of waveforms more likely represented the true cfPWV and the outlying scan was
discarded. This was based on the consistency and clarity of the scan. Technicians were trained
to obtain 3 scans within <1.0 m/s with quality waveforms and so consulting the waveforms due
to differences ≥1.0 m/s across scans during the same assessment was rarely needed (<5% of the
time). If it was unclear which scan(s) should be excluded based on visual inspection of the
waveform, then all scans are retained but the quality score is downgraded (see below).

After this, remaining scans were averaged for a single value of cfPWV. Quality was
assigned as ‘excellent’ if all scans contributing to the average have a standard deviation across
waveforms of <5% and the difference across individual scans was <0.5 m/s. Quality was
assigned as ‘good’ if any scan contributing to the average had error between 5%-10% and/or
there were absolute differences across scans that were 0.5-1.0 m/s. Quality was assigned as
‘moderate’ if all scans had <10% error but the absolute difference across scans was ≥1.0 m/s.

3.4.7.4 Reliability
The PAWMRC had 4 technicians conducting cfPWV testing for participants of the current study.
Intra-rater reliability (ICC) have ranged from 85%-91% in 2013-2014.

3.5 INCLUSION CRITERIA FOR DATA ANALYSES
The primary outcome in the current study was 6-month changes in cfPWV. Overall obesity
(blood weight, BMI, % total body fat, abdominal fat (kg), % abdominal fat, CRF and MVPA
were assessed as predictor variables. Completion of cfPWV assessment at 0 and 6-month
assessments with at least one scan of sufficient quality was required for data analyses, which was
determined by data processing procedures outlined in the previous section. Subjects missing
cfPWV at one or more time point were compared to subjects with both cfPWV measurements to
characterize potential selection into the sample. Subjects missing data for predictor variables
(e.g. MVPA) at either time point were only excluded for analyses including that particular
variable.

3.6 STATISTICAL ANALYSES

All statistical analyses were performed using Stata v. 13.1 (College Station, TX) and alpha was
set at <0.05. Descriptive statistics were examined for mean baseline characteristics (age, height,
body weight, BMI, resting heart rate, resting blood pressure, waist circumference, % total body
fat, abdominal fat (kg), % abdominal fat, relative VO\textsubscript{2}(85\%MHR), objectively-measured MVPA and
cfPWV). Subjects included or excluded from the sample were compared using independent t-
tests, Mann-Whitney U tests, or chi-square, as appropriate. Dependent t-tests were used to assess
6-month differences in cfPWV, overall and abdominal obesity variables, VO\textsubscript{2}(85\%MHR) and
objectively-measured MVPA. For specific aim 1, Pearson correlation coefficients were
calculated to determine the relationships between 6-month changes in cfPWV and the following
parameters: body weight, BMI, % total body fat, waist circumference, abdominal fat (kg), %
ambitional fat, relative VO\textsubscript{2}(85\%MHR), and measures of MVPA (minutes per week, MET-minutes
per week, minutes per week in 10-minute bouts and MET minutes per week in 10-minute bouts).
Scatterplots were generated to evaluate non-linear trends and potential points of influence (see
Appendix A).
To address specific aims 2 and 3, multiple linear regression was performed to determine the effect of changes in CRF and MVPA on cfPWV with changes in body weight and abdominal fat as covariates. First, age, gender, race, and smoking were considered as potentially confounding variables. Next, changes in body weight and abdominal fat were added individually as covariates to explore the independent effects of CRF and MVPA changes on cfPWV in the context of changes in overall and abdominal obesity. Baseline value was considered as an additional covariate for adjustment, but did not affect results and therefore, was not included for reasons of parsimony.

Exploratory analyses were conducted for gender differences and associations were evaluated after excluding men (N=15, 18%), who were fewer in the sample. Also, associations between changes in cfPWV and potentially mediating variables (systolic blood pressure, diastolic blood pressure, and resting heart rate) were evaluated. Lastly, changes in predictor variables across categories of changes in cfPWV were evaluated to isolate extreme groups and identify potential trends that were not linear.

### 3.7 POWER ANALYSIS

The primary aim of this study was to determine if 6-month changes in overall and abdominal obesity, CRF and MVPA were related to 6-month changes in cfPWV and if relationships between changes in CRF and MVPA with changes in cfPWV occurred independently of changes in overall and abdominal obesity. Based on a projected sample size of 75-90 subjects, with alpha at 0.05, this study had 80% power to detect a correlation ranging from 0.286-0.312. This was considered adequate because a previous study had detected correlations ranging from 0.360-
0.602 between changes in obesity metrics and cfPWV. It was expected that independent variables would be related and it would be overly conservative to make adjustment for multiple correlations at this stage. The impact of multiple comparisons was considered in sensitivity analyses.
4.0 RESULTS

The purpose of this study was to determine if 6-month changes in overall and abdominal obesity, CRF, and MVPA were related to 6-month changes in cfPWV in overweight and obese adults, and if relationships between changes in CRF and MVPA with cfPWV occurred independently of changes in overall and abdominal obesity. The study was a pretest-posttest design with assessments performed at months 0 and 6.

4.1 SUBJECTS

One hundred and twelve (N=112) subjects from the parent study underwent cfPWV testing at the baseline assessment. Of those, nineteen (N=19) did not have cfPWV captured due to technician difficulty. Of the ninety-three (N=93) subjects with baseline cfPWV, ten (N=10) did not have cfPWV captured at month 6. Seven were lost to follow-up (N=7) and three (N=3) were missing due to technician difficulty. Therefore, eighty three (N=83) overweight and obese adults participating in the Heart Health parent study, a randomized clinical trial, were included in analyses for the present study (Figure 2). Of those, eighty three (N=83) had assessment of overall obesity, abdominal obesity and relative VO2^(85%MHR) at baseline and 6-month assessments. Seventy-eight (N=78) had assessment of MVPA, which met previously established criteria, at baseline and 6-month assessments (see section 3.4.6).
112 Subjects tested for baseline cfPWV

93 Subjects with quality baseline cfPWV

19 Poor quality or missing cfPWV scans

10 Missing 6-month cfPWV
  7 – Lost to follow-up
  3 – Poor quality or missing scans

83 Subjects with quality baseline & 6-month cfPWV

83 Subjects with assessment of overall and abdominal obesity, and relative VO2(85%MHR) at baseline & 6-months

78 Subjects with assessment of quality MVPA at baseline & 6-months

Figure 2. Flowchart of Subject Inclusion
4.1.1 Quality of Carotid-Femoral Pulse Wave Velocity Scans

To be included in data analyses addressing the specific aims, subjects needed quality cfPWV scans at both 0 and 6 month assessments. At baseline, twenty-eight (N=28) scans were assigned as ‘excellent,’ fifty-three (N=53) were assigned as ‘good’ and two (N=2) were assigned as ‘moderate.’ At 6-months, thirty-seven (N=37) had scans assigned as ‘excellent’ and forty-four (N=44) had scans assigned as ‘good’ and two (N=2) were ‘moderate.’ Analyses were repeated after excluding moderate scans (see section 4.3.3.3).

4.1.2 Description of sample

Descriptive statistics are presented in Table 1. Baseline data of included subjects (N=83) were compared to excluded subjects (N=29) by independent t-tests for parametric data; Mann Whitney-U tests or chi-square tests were used for nonparametric data. All baseline variables were normally distributed with the exception of the MVPA variables. Excluded subjects were more likely to have higher body weight ($p=0.033$) and higher abdominal fat (kg) ($p=0.01$). This difference may be explained by missed or poor quality cfPWV scans in higher weight classes, which can be more difficult to capture.
<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of Included and Excluded Subjects</th>
<th>Included (N=83)</th>
<th>Excluded (N=29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td>0.645</td>
</tr>
<tr>
<td>Males</td>
<td>15 (18.0%)</td>
<td>6 (20.7%)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>68 (81.9%)</td>
<td>23 (79.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Race†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>56 (68.3%)</td>
<td>22 (78.6%)</td>
<td>0.264</td>
</tr>
<tr>
<td>Non-White</td>
<td>25 (30.5%)</td>
<td>6 (37.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Current Smoking</strong></td>
<td></td>
<td></td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>1 (1.2%)</td>
<td>2 (6.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>44.3 ± 7.9</td>
<td>45.1 ± 8.7</td>
<td>0.681</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>167.2 ± 7.4</td>
<td>168.6 ± 8.0</td>
<td>0.378</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>89.5 ± 13.4</td>
<td>96.1 ± 15.9</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>32.0 ± 3.9</td>
<td>33.7 ± 4.2</td>
<td>0.053</td>
</tr>
<tr>
<td><strong>Waist Circumference (cm)</strong></td>
<td>104.8 ± 8.8</td>
<td>108.6 ± 13.4</td>
<td>0.089</td>
</tr>
<tr>
<td><strong>Total Fat (%)</strong></td>
<td>43.0 ± 5.6</td>
<td>43.7 ± 5.9</td>
<td>0.538</td>
</tr>
<tr>
<td><strong>Abdominal Fat (kg)</strong></td>
<td>7.0 ± 1.5</td>
<td>7.8 ± 1.7</td>
<td>0.384</td>
</tr>
<tr>
<td><strong>Abdominal Fat (%)</strong></td>
<td>54.3 ± 6.1</td>
<td>44.6 ± 10.4</td>
<td>0.341</td>
</tr>
<tr>
<td><strong>Resting SBP (mmHg)†</strong></td>
<td>119.0 ± 11.0</td>
<td>121.4 ± 12.9</td>
<td>0.578</td>
</tr>
<tr>
<td><strong>Resting DBP (mmHg)</strong></td>
<td>70.8 ± 9.0</td>
<td>71.9 ± 9.2</td>
<td>0.533</td>
</tr>
<tr>
<td><strong>Resting Heart Rate (bpm)</strong></td>
<td>67.0 ± 9.9</td>
<td>65.7 ± 9.6</td>
<td>0.116</td>
</tr>
<tr>
<td><strong>VO₂(85%MHR) (ml/kg·min)</strong></td>
<td>22.1 ± 4.3</td>
<td>22.1 ± 3.3</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>MVPA (MET-min/wk)‡†</strong></td>
<td>837.0 (409.6, 1501.6)</td>
<td>661.8 (157.3, 1156.8)</td>
<td>0.081</td>
</tr>
<tr>
<td><strong>MVPA (MET-min/wk in 10 min bouts)‡†</strong></td>
<td>298.6 (74.6, 701.8)</td>
<td>88.2 (32.0, 1075.2)</td>
<td>0.096</td>
</tr>
<tr>
<td><strong>MVPA (min/wk)‡†</strong></td>
<td>252.5 (123, 440)</td>
<td>206 (47.3, 350)</td>
<td>0.090</td>
</tr>
<tr>
<td><strong>MVPA (min/wk in 10 min bouts)‡‡</strong></td>
<td>81.75 (21, 178)</td>
<td>24 (10, 140)</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>cfPWV (m/s)‡‡</strong></td>
<td>7.5 ± 1.2</td>
<td>7.1 ± 1.2‡‡</td>
<td>0.309</td>
</tr>
</tbody>
</table>

BMI: body mass index, cfPWV: carotid-femoral pulse wave velocity, DBP: diastolic blood pressure, MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, SBP: systolic blood pressure, VO₂(85%MHR): volume of oxygen at 85% age-predicted maximal heart rate

†Median (interquartile range) and Mann Whitney-U test used for nonparametric data

†1 subject missing

‡19 subjects missing
4.2 SIX-MONTH CHANGES

Eighty-three (N=83) subjects with baseline and 6-month cfPWV were included in the data analyses to address the specific aims. Only subjects with baseline and 6-month data for a given predictor variable were included in analysis of that particular variable and N values are noted when <83.

At 6 months, significant differences were observed for body weight, BMI, waist circumference, % total body fat, abdominal fat (kg), % abdominal fat, relative VO_2(85%MHR), all MVPA variables (N=78) and cfPWV (all p<0.001) (Table 2).
<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean ± SD</th>
<th>Month 6 Mean ± SD</th>
<th>Change Mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>89.5 ± 13.4</td>
<td>79.9 ± 14.0</td>
<td>-9.6 (5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.0 ± 3.9</td>
<td>28.5 ± 4.3</td>
<td>-3.5 (2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>104.8 ± 8.8</td>
<td>97.7 ± 11.0</td>
<td>-7.1 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Fat (%)</td>
<td>43.0 ± 5.6</td>
<td>37.5 ± 7.5</td>
<td>-5.5 ± 3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal Fat (kg)</td>
<td>7.0 ± 1.5</td>
<td>6.0 ± 1.5</td>
<td>-1.1 (7.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal Fat (%)</td>
<td>54.4 ± 6.1</td>
<td>44.6 ± 10.3</td>
<td>-9.8 (6.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO₂(85%MHR) (ml/kg/min)</td>
<td>22.1 ± 4.3</td>
<td>24.4 ± 5.9</td>
<td>2.3 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MVPA (MET-min/wk)</td>
<td>837 (410, 1502)†</td>
<td>1434 (898, 2599)†</td>
<td>682.2 (1075)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MVPA (MET-min/wk in 10 min bouts)</td>
<td>299 (75, 701)†</td>
<td>903 (279, 1506)†</td>
<td>636 (920)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MVPA (min/wk)</td>
<td>253 (123, 440)†</td>
<td>388 (255, 713)†</td>
<td>165.9 (285.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MVPA (min/wk in 10 min bouts)</td>
<td>82 (21, 178)†</td>
<td>216 (74, 361)†</td>
<td>131.4 (201.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cfPWV (m/s)</td>
<td>7.5 ± 1.2</td>
<td>7.1 ± 1.1</td>
<td>-0.5 (0.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI: body mass index, cfPWV: carotid-femoral pulse wave velocity, MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, VO₂(85%MHR): volume of oxygen at 85% age-predicted maximal heart rate
†Median (interquartile range) reported for nonparametric data
†5 subjects missing
4.2.1 Unadjusted Correlations of Changes in Overall and Abdominal Obesity, Cardiorespiratory Fitness and Moderate-Vigorous Physical Activity on Carotid-Femoral Pulse Wave Velocity

Pearson correlation coefficients (r) were calculated to determine the relationships between 6-month changes in cfPWV and changes in body weight, BMI, waist circumference, % total body fat, abdominal fat (kg), % abdominal fat, relative VO2(85%MHR) and MVPA variables (Table 3). A statistically significant, inverse correlation was found between cfPWV and relative VO2(85%MHR) (r=-0.221, p=0.045); all other overall and abdominal fatness variables and MVPA variables were not significantly correlated with cfPWV (p>0.05) (see Appendix A).

<table>
<thead>
<tr>
<th></th>
<th>Pearson’s r (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.124 (0.264)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.090 (0.416)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.177 (0.111)</td>
</tr>
<tr>
<td>Total Body Fat (%)</td>
<td>0.114 (0.335)</td>
</tr>
<tr>
<td>Abdominal Fat (kg)</td>
<td>0.174 (0.115)</td>
</tr>
<tr>
<td>Abdominal Fat (%)</td>
<td>0.112 (0.348)</td>
</tr>
<tr>
<td>VO2(85%MHR) (ml/kg·min)</td>
<td>-0.221 (0.045)</td>
</tr>
<tr>
<td>MVPA (MET-min/wk)†</td>
<td>-0.027 (0.812)</td>
</tr>
<tr>
<td>MVPA (MET-min/wk in 10 min bouts)†</td>
<td>-0.107 (0.351)</td>
</tr>
<tr>
<td>MVPA (min/wk)†</td>
<td>0.023 (0.846)</td>
</tr>
<tr>
<td>MVPA (min/wk in 10 min bouts)†</td>
<td>-0.036 (0.752)</td>
</tr>
</tbody>
</table>

BMI: body mass index, MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, VO2(85%MHR): volume of oxygen at 85% age-predicted maximal heart rate
†5 subjects missing
4.3 INDEPENDENT EFFECTS OF CARDORESPIRATORY FITNESS AND MODERATE-VIGOROUS PHYSICAL ACTIVITY ON CAROTID-FEMORAL PULSE WAVE VELOCITY

4.3.1 Relative VO$_2$(85%MHR)

Multiple linear regressions were used to evaluate associations between relative VO$_2$(85%MHR) and cfPWV with increasing covariate adjustment as follows: adjusted for demographics, adjusted for demographics and change in overall obesity, and adjusted for demographics and change in abdominal fat.

Of the demographic variables considered (age, gender, race and smoking), only age ($r$=-0.275, $p=0.012$) and gender (men: -0.7 m/s vs. women: -0.4 m/s, $p<0.001$) were found to be associated with change in cfPWV. Race was not related to change in cfPWV (white: -0.4 m/s vs. non-white: -0.5 m/s, $p=0.923$) and therefore was not included in subsequent analyses. Only one subject (N=1) in the sample reported smoking at baseline and no subjects (N=0) reported smoking at 6 months. Since smoking was a rare exposure, it was not included in subsequent analyses. Based on unadjusted correlations (see Table 3), body weight and abdominal fat (kg) had the highest correlations for overall obesity and abdominal obesity, respectively, and were thus used in multivariable models for specific aims 2 and 3.

Change in relative VO$_2$(85%MHR) alone had a statistically significant effect on cfPWV change ($\beta$=-0.036, $p=0.045$), but adjustment for demographic covariates (age and gender)
attenuated the relationship ($\beta=-0.030$, $p=0.090$) so that the effect of relative VO$_{2(85\%MHR)}$ was no longer statistically significant. With the addition of body weight and abdominal fat, the coefficient was unaffected ($\beta=-0.030$, $p=0.106$ and $\beta=-0.030$, $p=0.107$, respectively).

### 4.3.2 Moderate-Vigorous Physical Activity

To evaluate the effect of change in measures of MVPA on change in cfPWV, linear regression was used with progressive covariate adjustment as follows: adjusted for demographics, adjusted for demographics and change in body weight and adjusted for demographics and change in abdominal fat. For regression models, denominators were changed for MVPA variables to aid in interpretation. Estimated change in cfPWV ($\beta$) per MVPA minutes accumulated in one-minute bouts is reported per 60 minutes. Estimated change in cfPWV ($\beta$) per MVPA minutes accumulated in 10-minute bouts is reported per 60 minutes. Estimated change in cfPWV ($\beta$) per volume of MVPA accumulated in either 1-minute or 10-minute bouts is reported per 180 MET-minutes (60 minutes x 3 METS) None of the MVPA measures were associated with changes in cfPWV with or without covariate adjustment (Table 4).
### Table 4. Associations Between Changes in Relative VO$_2$(85%MHR) and Moderate-Vigorous Physical Activity with Carotid-Femoral Pulse-Wave Velocity

<table>
<thead>
<tr>
<th></th>
<th>Model 1 $\beta$</th>
<th>Model 2 $\beta$</th>
<th>Model 3 $\beta$</th>
<th>Model 4 $\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in VO$_2$(85%MHR) (ml/kg·min)</td>
<td>-0.036</td>
<td>p=0.045</td>
<td>-0.009</td>
<td>p=0.485</td>
</tr>
<tr>
<td>Change in MVPA (per 180 MET-min/wk)</td>
<td>-0.030</td>
<td>p=0.090</td>
<td>-0.015</td>
<td>p=0.801</td>
</tr>
<tr>
<td>Change in MVPA (per 180 MET-min/wk in 10 min bouts)</td>
<td>-0.001</td>
<td>p=0.892</td>
<td>-0.010</td>
<td>p=0.895</td>
</tr>
<tr>
<td>Change in MVPA (per 60 min/wk)</td>
<td>0.007</td>
<td>p=0.684</td>
<td>0.002</td>
<td>p=0.897</td>
</tr>
<tr>
<td>Change in MVPA (per 60 min/wk in 10 min bouts)</td>
<td>0.012</td>
<td>p=0.596</td>
<td>-0.010</td>
<td>p=0.675</td>
</tr>
</tbody>
</table>

MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, VO$_2$(85%MHR): volume of oxygen at 85% age-predicted maximal heart rate

1Model 1 unadjusted
2Model 2 adjusted for age and gender
3Model 3 adjusted for age, gender, and change in body weight (kg)
4Model 4 adjusted for age, gender, change in abdominal (kg)
55 subjects missing

### 4.3.3 Sensitivity and Exploratory Analyses

#### 4.3.3.1 Gender Differences

Sensitivity analyses were conducted to evaluate potential gender differences. Paired t-tests assessed 6-month changes within each gender and independent t-tests compared changes over time by gender. All 6-month variables were statistically different from those at baseline in females ($p<0.001$) and all except MVPA in minutes per week, minutes per week accumulated ion 10-minutes bouts and total MET-minutes per week and ($p>0.1$) were statistically different in males ($p<0.001$). Changes in % body weight, BMI, % abdominal fat, relative VO$_2$(85%MHR),
and all MVPA variables were not significantly different between genders (all $p>0.1$). Men had significantly greater decreases in cfPWV ($p=0.047$), body weight ($p=0.040$) and waist circumference ($p=0.030$). Associations were evaluated after excluding men, who were less of the sample (N=15). In the unadjusted model with men excluded, the correlation between the change in relative VO$_2$(85%MHR) and change in cfPWV was attenuated and no longer statistically significant ($r=-0.145, p=0.245$). The correlations between changes in all MVPA variables and change in cfPWV remained nonsignificant (all $p>0.05$).
### Table 5. Gender Differences for 6-Month Changes in Overall and Abdominal Obesity, Relative VO$_2$(85%MHR) Moderate-Vigorous Physical Activity and Carotid-Femoral Pulse-Wave Velocity

<table>
<thead>
<tr>
<th></th>
<th>Males (N=15)</th>
<th>Change Mean (SD)</th>
<th>Females (N=68)</th>
<th>Change Mean (SD)</th>
<th>p$^#$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>100.0 ± 13.3</td>
<td></td>
<td>87.1 ± 12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Months</td>
<td>87.5 ± 14.8</td>
<td>-12.6 (7.2)$^*$</td>
<td>78.2 ± 13.3</td>
<td>-9.0 (5.3)$^*$</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>BMI (kg/m$^2$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>31.8 ± 3.8</td>
<td></td>
<td>32.0 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>27.7 ± 4.2</td>
<td>-4.0 (2.3)$^*$</td>
<td>28.7 ± 4.3</td>
<td>-3.3 (2.0)$^*$</td>
<td>0.219</td>
</tr>
<tr>
<td><strong>Waist Circumference (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>110.0 ± 5.1</td>
<td></td>
<td>103.6 ± 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>98.8 ± 9.0</td>
<td>-11.3 (7.0)$^*$</td>
<td>97.4 ± 11.4</td>
<td>-6.2 (7.3)$^*$</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Abdominal Fat (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.5 ± 1.1</td>
<td></td>
<td>6.7 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>6.8 ± 1.4</td>
<td>-1.7 (0.9)</td>
<td>5.8 ± 1.4</td>
<td>-0.9 (0.6)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Abdominal Fat (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>49.8 ± 4.4</td>
<td></td>
<td>55.4 ± 6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>37.0 ± 10.6</td>
<td>-12.8 (8.6)$^*$</td>
<td>46.2 ± 9.6</td>
<td>-9.2 (6.1)$^*$</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>VO$_2$(85%MHR) (ml/kg·min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26.4 ± 3.2</td>
<td></td>
<td>21.1 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>30.1 ± 4.5</td>
<td>3.7 (3.2)$^*$</td>
<td>23.1 ± 5.5</td>
<td>1.9 (4.4)$^*$</td>
<td>0.145</td>
</tr>
<tr>
<td><strong>MVPA (MET min/wk)$^+$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1609 (1240, 3010)$^+$</td>
<td></td>
<td>653 (348, 1187)$^+$</td>
<td></td>
<td>0.454</td>
</tr>
<tr>
<td>6 Months</td>
<td>2971 (2084, 4007)$^+$</td>
<td></td>
<td>870.0 (1591.3)</td>
<td>1261 (562, 1956)$^+$</td>
<td></td>
</tr>
<tr>
<td><strong>MVPA (MET min/wk in 10 min bouts)$^+$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.776</td>
</tr>
<tr>
<td>Baseline</td>
<td>886 (342, 1632)$^+$</td>
<td></td>
<td>239 (56, 537)$^+$</td>
<td></td>
<td>0.461</td>
</tr>
<tr>
<td>6 Months</td>
<td>1720 (1236, 2776)$^+$</td>
<td></td>
<td>794.7 (1321.0)$^*$</td>
<td></td>
<td>0.776</td>
</tr>
<tr>
<td><strong>MVPA (min/wk)$^+$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>449 (343, 899)$^+$</td>
<td></td>
<td>197 (105, 349)$^+$</td>
<td></td>
<td>0.461</td>
</tr>
<tr>
<td>6 Months</td>
<td>725 (554, 1001)$^+$</td>
<td></td>
<td>184.9 (423.1)</td>
<td></td>
<td>0.776</td>
</tr>
<tr>
<td><strong>MVPA (min/wk in 10 min bouts)$^+$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.682</td>
</tr>
<tr>
<td>Baseline</td>
<td>224 (94, 382)$^+$</td>
<td></td>
<td>65 (17, 130)$^+$</td>
<td></td>
<td>0.682</td>
</tr>
<tr>
<td>6 Months</td>
<td>400 (325, 660)$^+$</td>
<td></td>
<td>150.7 (307.6)</td>
<td></td>
<td>0.682</td>
</tr>
<tr>
<td><strong>cfPWV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.1 ± 1.4</td>
<td></td>
<td>7.3 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Months</td>
<td>7.4 ± 1.0</td>
<td>-0.7 (0.8)$^*$</td>
<td>7.0 ± 1.1</td>
<td>-0.4 (0.6)$^*$</td>
<td>0.006</td>
</tr>
</tbody>
</table>

BMI: body mass index, MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, VO$_2$(85%MHR): volume of oxygen

$^+$Median (interquartile range) reported for nonparametric data

$^*$p<0.001 for 6-month change

$^\#$Comparison of change in males and females

$^\dagger$5 subjects missing

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4.3.3.2 Blood Pressure and Heart Rate

Due to a potentially mediating effect of changes in blood pressure and heart rate on cfPWV, relationships between systolic blood pressure (SBP), diastolic blood pressure (DBP) and resting heart rate (RHR) with cfPWV were further explored. SBP significantly decreased from baseline (119.0 ± 11.0 mmHg to 113.9 ± 11.7 mmHg), along with DBP (70.8 ± 9.0 mmHg to 68.6 ± 8.7 mmHg) and RHR (67.0 ± 9.9 bpm to 62.0 ± 8.0 bpm). The change in cfPWV was moderately correlated with the change in SBP (r=0.379, p<0.001) (Figure 3) and DBP (r=0.317, p=0.004) (Figure 4). Change in cfPWV was not correlated with change in RHR (r=0.1562, p=0.158).

Figure 3. Change in Carotid-Femoral Pulse Wave Velocity Related to Change in Systolic Blood Pressure
Figure 4. Change in Carotid-Femoral Pulse Wave Velocity Related to Change in Diastolic Blood Pressure

In addition, SBP, DBP, and RHR were evaluated as possible explanatory variables in linear regression with adjustment for age and gender. The relationship between change in relative VO₂(85%MHR) and change in cfPWV was slightly attenuated by the addition of change in SBP (β=−0.021, p=0.233) or change in DBP (β=−0.024, p=0.170) to the model, but was not affected by change in RHR (β=−0.029, p=0.100). The effect of all MVPA variables remained nonsignificant (all p>0.1) with the addition of SBP, DBP, and RHR, and changes in coefficients were <10% (data not shown).
4.3.3.3 Sensitivity analyses considering quality of outcome assessment and multiple comparisons

Four (N=4) cfPWV scans included in the analysis were of ‘moderate’ quality due to error rate <10% but absolute difference across scans were <1.0 m/s and, based on visual inspection of the original waveforms, no single scan met exclusionary criteria (see section 3.4.7.3). To further evaluate the influence of these lower quality scans, these scans were excluded and analyses were repeated. The results were unchanged (data not shown).

In addition, the impact of adjustment for multiple comparisons was considered. The only statistically significant relationship was observed between changes in relative $VO_{2(85%MHR)}$ and cfPWV ($r=-0.221$, $p=0.045$). With 10 independent predictors in Table 3, Bonferroni adjustment would change the estimated chance of type 1 error to $p=0.45$. Such adjustment is likely overly conservative but does highlight that the association we observed between changes in relative $VO_{2(85%MHR)}$ and cfPWV was of borderline statistical significance.

4.3.3.4 Analysis of nonlinear trends across categories of change in cfPWV

Changes in predictor variables across categories of change in cfPWV were evaluated to isolate extreme groups and identify potential trends that were not linear. Categories were based on a clinically significant change in cfPWV of 1 m/s: a decrease in cfPWV ≥1 m/s, a decrease in cfPWV between 0-1 m/s, or any increase (>0 m/s) in cfPWV. Across groups, subjects who had the greatest decreases in cfPWV (≥1 m/s) had the greatest decreases in body weight, abdominal fat and relative $VO_{2(85%MHR)}$ and the smallest increases in MVPA, though there were no significant trends in any predictor variable across groups. These findings are consistent with the correlations observed between the predictor variable and the change in cfPWV.
Table 6. Changes in Overall and Abdominal Obesity, Relative VO₂(85% MHR) and Moderate-Vigorous Physical Activity by Categories of Change in Carotid-Femoral Pulse Wave Velocity

<table>
<thead>
<tr>
<th></th>
<th>Decrease &gt;1 (N=15)</th>
<th>Decrease 0-1 (N=45)</th>
<th>Increase &gt;0 (N=23)</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Weight (kg)</td>
<td>-10.0 (5.0)</td>
<td>-9.8 (6.4)</td>
<td>-9.0 (5.3)</td>
<td>0.582</td>
</tr>
<tr>
<td>Change in BMI (kg/m²)</td>
<td>-3.5 (1.7)</td>
<td>-3.5 (2.2)</td>
<td>-3.3 (1.9)</td>
<td>0.720</td>
</tr>
<tr>
<td>Change in Waist Circumference cm</td>
<td>-7.9 (6.3)</td>
<td>-7.4 (7.8)</td>
<td>-6.1 (7.5)</td>
<td>0.426</td>
</tr>
<tr>
<td>Change in Total Fat (%)</td>
<td>-5.8 ± 4.2</td>
<td>-5.5 (3.8)</td>
<td>-5.2 (3.3)</td>
<td>0.635</td>
</tr>
<tr>
<td>Change in Abdominal Fat (kg)</td>
<td>-11.8 (7.4)</td>
<td>-10.7 (7.6)</td>
<td>-9.8 (6.5)</td>
<td>0.368</td>
</tr>
<tr>
<td>Change in Abdominal Fat (%)</td>
<td>-10.5 (8.0)</td>
<td>-9.8 (6.6)</td>
<td>-9.6 (6.3)</td>
<td>0.700</td>
</tr>
<tr>
<td>Change in VO₂(85%MHR) (ml/kg·min)</td>
<td>3.6 (4.3)</td>
<td>2.5 (4.5)</td>
<td>1.0 (4.4)</td>
<td>0.056</td>
</tr>
<tr>
<td>Change in MVPA (MET-min/wk)†</td>
<td>532.7 (1086.0)</td>
<td>672.2 (973.7)</td>
<td>808.9 (1059.9)</td>
<td>0.448</td>
</tr>
<tr>
<td>Change in MVPA (MET-min/wk in 10 min bouts)</td>
<td>451.9 (1130.4)</td>
<td>693.1 (883.9)</td>
<td>630.8 (886.4)</td>
<td>0.510</td>
</tr>
<tr>
<td>Change in MVPA (min/wk)†</td>
<td>114.7 (371.1)</td>
<td>160.0 (255.0)</td>
<td>214.2 (280.4)</td>
<td>0.297</td>
</tr>
<tr>
<td>Change in MVPA (min/wk in 10 min bouts)†</td>
<td>84.7 (250.4)</td>
<td>139.9 (184.4)</td>
<td>147.7 (200.0)</td>
<td>0.388</td>
</tr>
</tbody>
</table>

Data displayed as mean (SD).
MET: metabolic equivalent, MVPA: moderate-vigorous physical activity, VO₂(85%MHR): volume of oxygen
†5 subjects missing
5.0 DISCUSSION

5.1 SUMMARY OF THE MAIN FINDINGS

The purpose of this study was to determine if 6-month changes in overall and abdominal obesity, CRF and MVPA were related to 6-month changes in cfPWV in overweight and obese adults, and if the relationships between changes in CRF and MVPA with cfPWV occurred independently of changes in overall and abdominal obesity. This study is the first to assess the independent effects of fitness and objectively-measured MVPA on cfPWV with changes in weight and abdominal fat.

Over 6 months, subjects in this sample had highly significant changes in body weight, BMI, waist circumference, total body fat, abdominal fat, CRF and MVPA. In addition, clinically meaningful reductions in cfPWV were achieved (-0.5 m/s). Six-month changes in cfPWV showed a weak, inverse correlation with the change in relative VO$_2$(85%MHR) (fitness), but showed no association with changes in MVPA, body weight, abdominal fat, or other fatness variables. Though the association between change in fitness and change in cfPWV did not persist after adjustment for demographic variables, the lack of any association between measures of fatness and cfPWV suggest that the highly significant reductions in cfPWV observed in this study population were driven more by improved fitness than by changes in weight and abdominal fat loss.
5.2 ASSOCIATIONS BETWEEN CAROTID-FEMORAL PULSE WAVE VELOCITY AND COVARIATES

5.2.1 Body Weight and Abdominal Fat

Arterial stiffness is an independent risk factor for cardiovascular disease\(^{10,13}\) and has been related to higher overall and abdominal obesity in cross-sectional and longitudinal studies.\(^{11,25,26,54,144,145}\) Similarly, several lifestyle intervention studies have observed improvements in cfPWV in overweight and obese adults and some have shown positive associations between changes in arterial stiffness and obesity measures.\(^{42,43,134}\) Based on the body of literature, it was hypothesized that weight loss, abdominal fat loss and reductions in cfPWV would occur after 6 months of lifestyle intervention and that changes in these measures of fatness would be directly associated with the beneficial changes in cfPWV. An average weight loss of 9.6 kg (10.5%) along with a clinically meaningful change in cfPWV (-0.5 m/s) was observed in this study over 6 months. However, the changes in measures of obesity were not correlated with the change in cfPWV. These findings are consistent with some intervention studies,\(^{129,134}\) but conflict with others.\(^{42-44}\)

Hughes et al. reported a nonsignificant relationship between changes in body weight and cfPWV measured by tonometry over the first 6 months of the SAVE lifestyle intervention that included components of dietary restriction and physical activity.\(^{129}\) This study had a sample of 339 sedentary, overweight and obese adults (age: 37.9, BMI: 32.8 kg/m\(^2\)), who were otherwise healthy, but had a slightly higher cfPWV at baseline of 8.8 m/s in comparison to the 7.5 m/s observed in the current study. After 6 months, SAVE study subjects had significant reductions in body weight (-7.0 kg) and cfPWV (-0.5 m/s), but the authors stated that these changes were
The consistency of these findings with the current study strengthen the argument that amount of weight change may not explain positive cfPWV changes within the context of a lifestyle invention in an uncomplicated overweight/obese adult population over 6 months.

Interestingly, in a later publication of the same cohort, Cooper et al. found that after 12 months of behavioral weight loss intervention, the change in BMI was related to the change in cfPWV. It is not clear why weight change was related to cfPWV at 12 months and not at 6 months in SAVE, but this contrasting finding could reflect a greater variability in response at 12 months or a longer time course needed for adaptation.

The current study finding that weight loss was not associated with the change in cfPWV is also consistent with results from Nordstrand et al., who assessed cfPWV by tonometry in 179 morbidly obese subjects (age: 45.2, BMI: 42.5 kg/m²) as part of a 7-week non-randomized trial. Individuals in a lifestyle intervention (calorie restriction and 180 minutes of supervised MVPA for 3 days per week) were compared to a low-calorie, diet-only group (900 kcal/day). After 7 weeks, weight significantly decreased in both groups and weight loss was significantly greater in the low-calorie group (-9.4 vs. -6.6 kg, \( p = 0.006 \)). However, the low-calorie diet group did not improve cfPWV, while the lifestyle intervention group showed significant reductions in cfPWV (diet only: -0.2 m/s; lifestyle: -0.6 m/s, \( p = 0.004 \)). Among all participants, and similar to the findings of the current study, there was not a significant correlation between weight loss and the change in cfPWV (\( r = -0.017, p = 0.821 \)). Despite major contrasts with the current study including shorter study length with more extreme interventions, heavier baseline BMI, higher baseline cfPWV (8.6 m/s) and medications for co-morbidities, the lacking relationship between
weight loss and cfPWV along with an absent change in cfPWV with diet-only group, are consistent with the current study’s results.

Other studies that have assessed the relationship between weight loss and arterial stiffness within lifestyle interventions are in disagreement with the finding from the current study. Goldberg et al. observed a significant correlation ($r=-0.549, p=0.02$) with weight loss and changes in large artery elasticity index (a measure of arterial compliance, reciprocal to arterial stiffness) in 37 obese subjects with metabolic syndrome after a 6-month intervention, identical to the duration of the current study. Furthermore, this group saw the greatest magnitude of compliancy change in those losing >5% of their initial body weight compared to those who lost <5%. The intervention included dietary restriction and one-hour of supervised aerobic exercise once per week. In addition to this session, MVPA was encouraged at least 3 days per week, but evaluation of MVPA was not reported. When compared to the current study, subjects were older (age: 55.3) and had metabolic disease, but were comparable in baseline BMI (36.1 kg/m²) and weight loss (-8.3 kg).

Dengo et al. found correlations between change in cfPWV and changes in total and abdominal fat over a 3-month weight loss intervention in 36 older, obese adults (age: 61.2, BMI: 30.0 kg/m²). Baseline body fat was similar to the current study sample (40.7%) and baseline cfPWV was slightly higher (8.2 m/s). Intervention components included a hypocaloric diet while maintaining (but not increasing) physical activity levels. The study also included a control group. Dengo et al. and the current study observed similar changes in total fat (-8% and -10%, respectively) and abdominal fat (-14% and -18%, respectively). Yet, unlike the current study, changes in weight and BMI were highly correlated with change in cfPWV ($r=0.602, p<0.05$; $r=0.616, p<0.05$) and change in abdominal fat was moderately correlated ($r=0.461, p<0.05$). The
older age of the Dengo et al. study population could have contributed to the qualitatively different associations. Another possible explanation is that the presence of a traditional control group could have contributed to the ability to find a statistical relationship between weight loss and decreased cfPWV.

Although the hypothesis that changes in body weight and abdominal fat would be associated with the change in cfPWV was rejected, the results of the current study are similar to several previous behavioral intervention studies. Differences in age, study duration and study design (e.g. utilizing a traditional control group) could explain why other studies found relationships between decreases in measures of obesity and decreases in cfPWV. Future research should evaluate these differences to determine if any are important to consider when interpreting the relationship of weight loss and cfPWV change.

5.2.2 Moderate-Vigorous Physical Activity and Cardiorespiratory Fitness

Higher levels of CRF and MVPA have been related to lower arterial stiffness in younger and older adults and in men and women,\(^6,64,146\) and these results occur independently of differences in body composition cross-sectionally.\(^{133}\) Physical activity intervention studies have typically evaluated the influence of MVPA on cfPWV (or related measures, e.g. arterial compliance), sometimes including fitness assessment. These studies have often found a beneficial effect of exercise training,\(^{56,64,133,134}\) though fewer have not supported the relationship\(^{130}\) or did not evaluate effects independently of weight loss.\(^{45}\) The current lifestyle intervention study observed highly significant increases in CRF and MVPA at 6 months. Consistent with the available literature for CRF\(^{45,70}\), the current study found that changes in \(\text{VO}_2^{(85\%\text{MHR})}\) were inversely,
although modestly, associated with changes in cfPWV. In contrast with previous research, the current study found that changes in MVPA were not related to changes in cfPWV.\textsuperscript{56,134}

Several short-term, supervised aerobic exercise training studies, mainly in obese, hypertensive individuals,\textsuperscript{133,134,147} have observed decreased cfPWV in a time frame of 4-12 weeks with intensities of 65 to 75\% of VO$_{2\text{max}}$. For example, a 4-week study from Collier et al. evaluated cfPWV by tonometry in 30 overweight adults (age: 49.8, BMI: 29.4 kg/m$^2$) with pre- or stage one hypertension.\textsuperscript{133} Within this study, one group performed supervised aerobic exercise at 65\% of VO$_{2\text{max}}$, 3 days per week. Significant reductions were observed for cfPWV (-1.0 m/s, ($p$=0.002), however subjects had substantially higher baseline cfPWV (12.1 m/s) compared to the current sample. In addition, the study subjects from Collier et al. had higher blood pressure at baseline and significant decreases in blood pressure, which could be explanatory in the observed cfPWV change. In a similar study in 40 older adults (age: 71.2) with hypertension, type II diabetes and hyperlipidemia, Madden and colleagues assessed cfPWV by tonometry after 3 months of supervised exercise sessions performed 3 days per week at 60-75\% of heart rate reserve.\textsuperscript{147} cfPWV was reduced by 14\% (12.0 m/s to 10.3 m/s) and no significant improvements in fitness or blood pressure were observed, but the supervised exercise and older population with preexisting conditions could explain why the current study observed different results. The results of these training studies do not reconcile with the findings of the current study, that MVPA was not associated with changes in cfPWV, but had major methodological differences including a shorter timeline, a consistent dose of MVPA which did not allow for analysis of correlations, preexisting conditions which are associated with arterial stiffness and the laboratory setting.
In contrast, Havlik et al. evaluated changes in cfPWV measured by ultrasound among 258 sedentary adults ages 35-75 who received a 24-month, home-based intervention to increase MVPA in the form of walking. At 6 months, self-reported MVPA was significantly increased (1.61 h/day to 2.00 h/day, \( p<0.001 \)) and was accompanied by a nonsignificant increase in cfPWV (0.26 m/s, \( p>0.05 \)), but no association was observed between the changes in cfPWV and MVPA at this time point. At 24 months, walking remained significantly increased from baseline (2.03 h/day), but a highly significant reduction in cfPWV was observed (-1.4 m/s, \( p<0.001 \)) and every one-hour increase in walking was associated with a -1.5 m/s reduction in cfPWV (\( p=0.013 \)). Of note, no significant weight loss was observed in the study. These results are comparable to the current study in that increased moderate activity (e.g. walking) was not associated with 6-month changes in cfPWV. The significant effect seen at 24-months may suggest that moderate activity takes a longer period of time to influence arterial stiffness.

Only a handful of intervention studies have assessed the independent effect of MVPA on cfPWV in the context of weight loss. A 7-week, nonrandomized weight loss intervention trial in morbidly obese subjects by Nordstrand et al. (mentioned above in section 5.2.1) found that only the lifestyle group (caloric restriction + supervised exercise for 180 minutes per day on 3 days per week) achieved improvements in cfPWV, while the low-calorie diet-only group did not. These results suggest that MVPA was a necessary component beyond weight loss to positively affect cfPWV. The results of the current study suggesting that change in MVPA does not have a relationship with change in cfPWV may differ because of the intense, supervised exercise stimulus and higher baseline BMI in the Nordstrand et al. study population.

In another weight loss study, Balkenstein et al. studied 37 men (age: 37, BMI: 32.3 kg/m²) over 3 months of energy restriction with or without the addition of exercise training at 40% of
VO2max to determine the effects on arterial compliance.130 Neither condition produced significant changes in arterial compliance and the authors concluded that no additional benefit of exercise could be supported. Though an overall effect was not observed, these findings are consistent with the current study and with the above-mentioned study by Havlik et al.,56 both suggesting that home-based, low-intensity (37 to 45% of VO2max) or moderate-intensity (46 to 63% of VO2max) physical activity interventions over 3-6 months may not reduce arterial stiffness in overweight and obese subjects who are otherwise healthy.

It is possible that the 6-month duration and recommendation to engage in MVPA (e.g. brisk walking) in the current study was not sufficient to observe an effect on cfPWV. Alternatively, vigorous-intensity physical activity (64 to 90% of VO2max) may be needed for vascular adaptation, particularly in shorter interventions.64,134 That vigorous-intensity physical activity might be important for improving arterial stiffness is supported by Boreham et al., who looked cross-sectionally at the influence of sport and leisure physical activity and fitness on arterial stiffness.68 They found that only sport activity, which is of greater intensity than leisure activity, was associated with cfPWV and the relationship was mediated by higher CRF. Furthermore, they found that these associations were independent of body fatness variables, which is in line with results of the current study.

Although the 6-month increases in MVPA in the current study did not show an effect on cfPWV, specific aspects of MVPA may still be important for improving arterial stiffness. The MVPA variables investigated in the current study were absolute duration of MVPA, absolute duration of MVPA accumulated in 10-minute bouts, and volume of MVPA (duration x intensity). Moderate and vigorous minutes of physical activity were not investigated separately
in this analysis. It is possible that combining moderate with vigorous physical activity could have obscured relationships between changes in vigorous physical activity and cfPWV.

No published reports have evaluated correlations between changes in fitness and changes in cfPWV in a lifestyle intervention trial, independent of weight loss. Blumenthal et al. saw a 19% increase in relative VO$_2$(85%MHR) in weight management subjects in 4 months, as well as improvements in cfPWV and a 9% weight loss. $^{45}$ These subjects attended supervised exercise sessions 3 days per week and were compared to a control group with no exercise. Subjects in this study had a baseline VO$_2$(85%MHR) similar to the current study sample (23.4 ml/kg·min vs. 22.1 ml/kg·min, respectively). Subjects with the added exercise intervention had the most weight loss, the greatest fitness gains and a greater reduction in cfPWV when compared to a diet-only control group. However, independent relationships of fitness and weight loss were not assessed. It should be noted that this study assessed cfPWV as a secondary outcome to blood pressure change and the relationship between change in fitness and cfPWV was not evaluated. The authors suggested that the improvement in fitness could have elicited additional benefit on stiffness beyond weight loss and blood pressure reduction, but did not evaluate these relationships analytically.

The current study observed a 10% fitness gain, just over a 10% weight loss and a 5% improvement in cfPWV. These analyses indicated a small effect of fitness change on cfPWV improvement, and the magnitude of this relationship was not altered by adjustment for changes in weight or abdominal fat. However, the change in fitness explained only a small portion of the improvement in cfPWV. Experts believe that the physiological benefits of CRF on cfPWV result primarily from increases in exercise training. $^{128}$ Primary mechanisms are thought to be a blood flow increase during exercise to stimulate nitric oxide from the endothelium $^{148}$, chronic
adaptations to shear stress, and improvements in vascular smooth muscle.\textsuperscript{64,149} Metabolic factors, such as insulin sensitivity and inflammatory response have also been postulated.\textsuperscript{150} Adaptations have generally been explained as both structural and functional. Structurally, repetitive bodily movements protect elastin fibers of the artery while limiting the promotion of collagen. From a functional perspective, regular training elicits a heightened chronic parasympathetic tone, resulting from increases in vasodilatory activity induced by nitric oxide.\textsuperscript{128,151} Although the effect of MVPA was not significant in this study, it may be of value for researchers to measure whether these specific intermediate physiological effects explain relationships between increased fitness or MVPA and improved cfPWV in future studies.

### 5.3 EFFECT OF POTENTIAL EXPLANATORY VARIABLES ON CAROTID-FEMORAL PULSE WAVE VELOCITY

#### 5.3.1 Demographic Variables

Associations with change in cfPWV were computed for demographic variables and were found to be statistically significant with age and gender, but not race. Smoking a rare occurrence (N=1) and did not affect any results. When adjusted for age and gender, the effect of changes in CRF on changes in cfPWV did not remain statistically significant but the coefficient was only somewhat attenuated (-17%). To further explore this finding, gender differences were evaluated and men were found to have significantly greater decreases in cfPWV. Changes in VO\textsubscript{2}(85\%MHR) and all MVPA variables were not found to be different between genders, but this could be in part
due to the small sample of men in the study (N=15, 16%). Future research should include a larger sample of men to more definitively address gender differences regarding cfPWV.

5.3.2 Cardiovascular Variables

In addition to cfPWV, the primary outcome in this study, other cardiovascular variables including blood pressure and heart rate were assessed. The variables were treated as having potentially mediating effects on relationships between changes in fatness, CRF, and MVPA with changes in cfPWV. As expected, a strong relationship was observed between change in cfPWV and changes in blood pressure. Furthermore, the effect of fitness was further attenuated and not statistically significant with the independent additions of systolic or diastolic blood pressure. This suggests that changes in blood pressure could mediate beneficial effects of increased CRF on cfPWV.

5.4 CLINICAL IMPLICATIONS

Carotid-femoral pulse wave velocity, the gold standard measurement of arterial stiffness, has been established as a precursor to chronic cardiovascular outcomes, such as hypertension and heart failure. Obesity, low CRF and low levels of MVPA are risk factors that are thought to exacerbate the progression of arterial stiffening and cardiovascular disease. Lifestyle interventions often target some or all of these components through weight management and physical activity strategies, but the current evidence is unclear with regard to which components are most effective to improve arterial health. Although this study did not show a statistically
significant effect of changes in fatness variables or MVPA on cfPWV, increased fitness emerged as a potentially important target to improve this aspect of cardiovascular health.

5.5 LIMITATIONS AND FUTURE RESEARCH

This study was designed to examine the effect of changes in overall and abdominal obesity, CRF and MVPA on changes in cfPWV in subjects enrolled in a standard behavioral weight loss intervention. A strength of this study was the use of gold standard measurements to evaluate abdominal obesity (DXA), CRF (graded exercise testing), MVPA (objective-measure) and arterial stiffness (cfPWV). Additionally, the sample was sedentary at baseline, free of disease and overall non-smokers, which limited confounding variables.

However, several limitations may have impacted results, and outcomes should be interpreted accordingly.

1) This study included obese (25.0-39.9 kg/m²), sedentary, otherwise healthy adults ages 18-55. Therefore, caution should be used when generalizing these findings to other populations, such as normal weight, habitually active or diseased.

2) This study was not designed to look at any differences in randomized groups that were established within the design of the parent study. Therefore, conclusions cannot be drawn on the effectiveness of varying doses of exercise on the variables of interest. The ability to assess such differences will occur upon completion of the parent study.

3) While the study did assess abdominal fat changes, separation of visceral and subcutaneous abdominal fat was not within the means of this study. Since visceral fat
is more closely related to cardiovascular outcomes, it may be valuable for future studies to assess this distinction in relation to cfPWV.

4) The sample size of the study was small and further research with larger samples could better characterize the relationships assessed in this study. The study was also not powered to detect small associations, adjust for many covariates or test for interaction.

5) Moderate and vigorous physical activity was not assessed independently in this study. Due to the potential for more vigorous physical activity to stimulate vascular adaptation, future research should distinguish the effects of varying intensities on cfPWV in the context of this study.

6) The 6-month duration of the study may not have been substantial enough to observe an effect of weight loss or MVPA that was observed in longer studies (12-24 months) and future intervention studies should consider this when assessing cfPWV.

7) A certain degree of testing bias was possible in the current study. Subjects measured at baseline assessment were overall unfamiliar with testing procedures, specifically cfPWV, which could have stimulated feelings of nervousness or unease and some elevation of cardiovascular parameters.
5.6 FINAL CONCLUSIONS AND FUTURE RESEARCH

Based on the results of the current study and the body of previous literature, several research gaps would be valuable for consideration in future studies. The primary questions raised from this study are: 1) why were the significant decreases in cfPWV at 6 months not explained by primary intervention components (weight loss and MVPA) and only marginally by fitness? and 2) why weren’t the expected relationships between changes in obesity, fitness and MVPA observed?

The first question suggests an influence of additional factors which affect cfPWV and could be measured in future research. While this study evaluated the chronic effect of MVPA on cfPWV, the acute effect may be important to consider. This research group recently found cfPWV to be significantly reduced 24 hours after a single session of MVPA. In the current study, subjects were sedentary at baseline assessment, but may have exercised the day prior to 6-month assessment and experienced a variability in response. Future studies could assess follow-up cfPWV after prior days with and without MVPA to elucidate the potential effects of acute as well as chronic MVPA on cfPWV. Other influences of the lifestyle intervention, for example improved diet quality or decreased salt intake, were not evaluated in the current study but could have contributed to a decline in cfPWV over the first 6 months and should also be considered in future studies.

With regard to the second question of why expected relationships were not observed, it is possible that follow-up was too brief for chronic physiological adaptations of the vasculature after weight loss or with a home-based MVPA to become apparent. Future studies of longer duration will be critical to understand if and how lifestyle intervention can improve arterial stiffness. Lastly, although this study did utilize an objective-measure of MVPA, the activity
monitor was worn for a period of one week, and in some cases, may not have been an accurate representation of normal behavior. Larger sample sizes might be needed to overcome the added variability in MVPA assessment.
Figure 5. Scatterplot of Change in Body Weight with Change in Carotid-Femoral Pulse Wave Velocity
Figure 6. Scatterplot of Change in Body Mass Index with Change in Carotid-Femoral Pulse Wave Velocity

Figure 7. Scatterplot of Change in Waist Circumference with Change in Carotid-Femoral Pulse Wave Velocity
Figure 8. Scatterplot of Change in % Total Body Fat with Change in Carotid-Femoral Pulse Wave Velocity

Figure 9. Scatterplot of Change in Abdominal Fat (kg) with Change in Carotid-Femoral Pulse Wave Velocity
Figure 10. Scatterplot of Change in % Abdominal Fat with Change in Carotid-Femoral Pulse Wave Velocity

Figure 11. Scatterplot of Change in Relative VO\(_2\)\(_{(85\%\text{MHR})}\) with Change in Carotid-Femoral Pulse Wave Velocity
Figure 12. Scatterplot of Change in Moderate-Vigorous Physical Activity (minutes per week) with Change in Carotid-Femoral Pulse Wave Velocity

Figure 13. Scatterplot of Change in Moderate-Vigorous Physical Activity (MET-minutes/week) with Change in Carotid-Femoral Pulse Wave Velocity
Figure 14. Scatterplot of Change in Moderate-Vigorous Physical Activity (minutes per week in 10 minute bouts) with Change in Carotid-Femoral Pulse Wave Velocity

Figure 15. Scatterplot of Change in Moderate-Vigorous Physical Activity (MET-minutes per week in 10 minute bouts) with Change in Carotid-Femoral Pulse Wave Velocity


