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## The Effects of Aerobic Exercise Training on Arterial Stiffness in the Metabolic Syndrome

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# **The Effects of Aerobic Exercise Training on Arterial Stiffness in the Metabolic Syndrome**

Brian Lee Reger

**Thesis submitted to the  
College of Medicine at West Virginia University  
in partial fulfillment of the requirements for the degree of**

Master of Science in Exercise Physiology

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2012

Keywords: metabolic syndrome, elasticity, lifestyle, arteries, aortic stiffness

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# Abstract

The Effects of Aerobic Exercise Training on  
Arterial Stiffness in the Metabolic Syndrome

**Brian L. Reger**

**Background:** Arterial dysfunction, due in part to arterial stiffening, is recognized as a surrogate end point for cardiovascular disease (CVD) and predicts the risk of future CV events. The metabolic syndrome (MetS) is a clustering of risk factors associated with increased stiffening and carotid artery thickening. Aerobic exercise is a well-established therapy that reduces CV events, and has proven to be an effective intervention against arterial stiffening and pathological wall remodeling in the healthy, hypertensive, and diabetic population.

**Aims:** The purpose of this thesis was to examine the impact of aerobic exercise training on arterial stiffness and wall remodeling in MetS individuals. We hypothesized that an 8-week exercise intervention would decrease stiffness and reduce carotid wall thickness, compared to inactive MetS participants.

**Methods:** We compared arterial stiffness (pulse wave velocity: PWV), carotid wall thickness (cIMT), central pulse pressure (cPP), and peak aerobic capacity ( $VO_2$ peak) between healthy subjects ( $n=15$ ) and MetS individuals ( $n=21$ ), free of CV events. Further, we examined how 8 weeks of aerobic exercise training in MetS individuals ( $n=11$ ) altered the above parameters, compared to MetS individuals ( $n=9$ ) who remained inactive. Two MetS participants did not finish the 8-week intervention and were only included in the baseline analysis. Cross-sectional comparisons were analyzed using two-tailed independent t-tests. The intervention data was analyzed using repeated measures analysis of variance.

**Results:** Carotid-femoral PWV (PWVcf) and cPP were 26% ( $P=0.001$ ) and 33% ( $p<0.05$ ) higher, respectively; in MetS individuals compared to healthy controls, and cIMT was 13% ( $p<0.05$ ) greater in the MetS group. There was no significant difference between the trained and non-trained group in all baseline indexes. The major finding of this study was that 8 weeks of aerobic exercise significantly attenuated PWVcf ( $8.1 \pm 0.5$  vs.  $7.2 \pm 0.4$  m/s,  $p<0.05$ ) in the trained group, and no change was observed in the non-trained group ( $8.0 \pm 0.5$  vs.  $7.9 \pm 0.3$  m/s,  $p=0.51$ ). Carotid IMT did not change in the trained or non-trained group ( $0.58 \pm 0.03$  vs.  $0.58 \pm 0.03$  mm,  $p=0.78$  and  $0.59 \pm 0.05$  vs.  $0.61 \pm 0.05$  mm,  $p=0.92$ ). Another discovery was the trained group increased  $VO_2$ peak ( $16.0 \pm 1.4$  vs.  $18.2 \pm 1.7$  ml/kg/min,  $p=0.05$ ), and no change was seen in the non-trained group ( $19.0 \pm 1.9$  vs.  $18.7 \pm 1.3$  ml/kg/min,  $p=0.76$ ). Further, PWVcf and cPP were both negatively correlated ( $r=-.40$  and  $r=-.51$ ,  $p<0.01$  for both) with  $VO_2$ peak.

**Conclusions:** Although the mechanisms underlying the improvement in arterial elasticity are not fully understood, there is strong evidence supporting an association with the improvement in peak aerobic capacity. Cross-sectional examination revealed increased stiffening and blunted  $VO_2$ peak in the MetS individuals. Negative correlation was established between composite measures of stiffness and  $VO_2$ peak. Following the aerobic exercise intervention, the trained group improved cardiorespiratory fitness, while reducing arterial stiffness. These are novel findings that indicate chronic, aerobic exercise is an effective therapeutic intervention that decreases arterial stiffness, but does not attenuate carotid wall thickening in the MetS.

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This work was conducted within the Exercise Physiology Department,  
West Virginia University, Morgantown, WV

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## Abbreviations

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|                       |  |
|-----------------------|--|
| AGEs                  | Advanced Glycation End Products                                      |
| Alx                   | Augmentation Index   |
| Alx@75                | Augmentation Index Corrected At Heart Rate of 75                     |
| AP                    | Augmentation Pressure  |
| bPP                   | Brachial Pulse Pressure  |
| BP                    | Blood Pressure   |
| BMI                   | Body Mass Index  |
| CAD                   | Coronary Artery Disease  |
| cPP                   | Central Pulse Pressure   |
| CVD                   | Cardiovascular Disease   |
| ECG                   | Electrocardiography  |
| EGIR                  | The European Group for the Study of Insulin Resistance               |
| FMD                   | Flow-Mediated Dilation   |
| FMDdia                | Flow-Mediated Dilation Percent Change in Diameter                    |
| FMDflow               | Flow-Mediated Dilation Percent Change in Flow                        |
| FMD:SR <sub>AUC</sub> | Flow-Mediated Dilation Normalized to Shear Rate Area Under the Curve |
| HDL                   | High-Density Lipoprotein   |
| HR                    | Heart Rate   |
| IDF                   | International Diabetes Foundation                                    |
| IMT                   | Intima-Media Thickness   |
| IMTavg                | Average Intima-Media Thickness (measured in one cardiac cycle)       |

## Abbreviations continued

---

|                     |   |
|---------------------|---|
| IMT <sub>max</sub>  | Maximum Intima-Media Thickness (measured in cardiac one cycle)    |
| LV                  | Left Ventricle  |
| LVH                 | Left Ventricular Hypertrophy                                      |
| MAP                 | Mean Arterial Pressure  |
| MetS                | Metabolic Syndrome  |
| MMPs                | Metalloproteinases  |
| MRS                 | Metabolic Risk Score  |
| NCEP ATP III        | National Cholesterol Education Program: Adult Treatment Panel III |
| NO                  | Nitric Oxide  |
| PP                  | Pulse Pressure  |
| PWA                 | Pulse Wave Analysis (Applanation Tonometry)                       |
| PWV                 | Pulse Wave Velocity   |
| PWV <sub>cf</sub>   | Carotid-Femoral Pulse Wave Velocity                               |
| PWV <sub>cr</sub>   | Carotid-Radial Pulse Wave Velocity                                |
| SR <sub>AUC</sub>   | Shear Rate Area Under The Curve                                   |
| VO <sub>2peak</sub> | Peak Aerobic Capacity   |
| VSMC                | Vascular Smooth Muscle Cell                                       |
| WHO                 | World Health Organization   |

# **Chapter 1**

## **Introduction**

## 1.1. Purpose

Arterial stiffness is a term used to describe the elasticity and rigidity of the arterial wall and is commonly caused by fracture and fragmentation of elastic lamellae, increased collagen concentration, vascular smooth muscle cell (VSMC) hypertrophy, and endothelial dysfunction (responsible for increased VSMC tone) [1-4]. The main function of the central arteries is to act both as a conduit, delivering blood to the peripheral tissue, and a cushion, buffering pulsatile ejection of blood from the heart in order to provide constant flow to organs [5]. In a stiffened artery the buffering capacity is attenuated, resulting in an increased risk for cardiovascular disease (CVD) [6, 7]. The metabolic syndrome (MetS) is a clustering of risk factors associated with the increased risk of CVD [8]. These risk factors include; elevated blood pressure (BP), high triglycerides, low high-density lipoprotein (HDL) cholesterol, hyperglycemia, and abdominal obesity. The MetS is associated with increased arterial stiffening [8, 9]. In addition, carotid intima-media thickening (IMT) is linked to arterial stiffness [10], and MetS individuals have been shown to have increased carotid thickening [11].

The purpose of this thesis is to examine the impact of aerobic exercise training on arterial stiffness and wall remodeling in individuals with the MetS. It has been shown previously that aerobic training in older, healthy people (previously sedentary) and hypertensive subjects can attenuate stiffening [12, 13]. It is also our goal to evaluate the effect of aerobic exercise training on carotid IMT in MetS individuals. Aerobic exercise intervention improved endothelial function and

reduces IMT in older, previously sedentary adults [14]. However, no study has examined the effect of exercise on carotid IMT in MetS individuals.

## **1.2. Specific Aims & Hypothesis**

Specific Aim #1: Determine the effect of 8 weeks of aerobic exercise training on arterial stiffness in individuals with the metabolic syndrome.

- Hypothesis #1: Individuals with the metabolic syndrome who exercise for 8 weeks will exhibit a decrease in arterial stiffening, compared to inactive participants.

Specific Aim #2: Determine the effect of 8 weeks of aerobic training on carotid intima-media thickening in individuals with the metabolic syndrome.

- Hypothesis #2: Individuals with the metabolic syndrome who exercise for 8 weeks will exhibit a decrease in carotid intima-media thickening, compared to inactive participants.

## **1.3. Background and Significance**

CVD is the leading cause of morbidity and mortality in modern societies, responsible for 2.4 million deaths every year [15]. People with the MetS are 3 times more likely to die of heart disease than their healthy counterparts [16]. It is estimated that one-fourth of the American population, over the age of 18, have the MetS [17]. MetS is defined by the presence of three or more of the following traits; abdominal obesity, low HDL cholesterol, elevated triglycerides, hyperglycemia, and hypertension [18].

Arterial stiffening, known as arteriosclerosis, is defined by lumen enlargement with wall thickening (remodeling) and a reduction of elastic properties (stiffening) [10]. It is a natural consequence of aging due to repetitive strain imposed on the arteries, and is increasingly recognized as a surrogate end point for CVD [19].

Arterial stiffness is increased in Mets individuals [20, 21], which may explain the increase in CVD associated with this syndrome [22]. Following ejection of blood from the heart, a pressure wave precedes the flow of blood and in a perfectly elastic aorta the reflected wave returns to the heart during diastole, when the aortic valve is closed [6]. This minimizes the pressure load imposed on the heart and increases pressure during diastole, when the coronary arteries are perfused [6]. The cushioning action of the central arteries is impaired in a stiffened artery, increasing the workload on the left ventricle (LV) and decreasing the efficient relationship between the arteries and heart [6]. Progressive stiffening of the arteries can occur with advancing age, mainly due to the structural and functional changes of the central arteries [5, 23].

Central pulse wave velocity (PWV), using applanation tonometry, is considered to be the gold standard for assessing arterial stiffness, non-invasively, and has been shown to be predictive of CV events [24]. PWV represents the time taken by the pulse to travel between two points of known distance, usually the carotid and femoral arteries [10]. In addition, evaluation of central pulse pressure [(cPP) (systolic BP (SBP) – diastolic BP (DBP))] and augmentation (Alx) provides an assessment of arterial stiffness, representing the cushioning capacity of the arteries and timing of the wave reflections, respectively [25]. Increasing SBP as a result of a stiffened vessel, results in an increase in cPP due to less elastin and more collagen activated at higher dispensability pressures. Hypertension, a MetS component, places an increased strain on central arteries through arterial remodeling [5].



Elastin fiber fracture and fatigue is a structural disturbance observed in a stiffened artery [6]. In a healthy, young person central arteries are composed of more elastin content, relative to collagen, enhancing the cushioning of blood flow and reflective waves toward the heart [26]. With aging, repetitive contractile strain reduces the amount of elastin and increases less distensible, collagenous fibers, in the proximal arteries [26]. VSMC hypertrophy, an increase in cell size, and increases in advanced glycation end products (AGEs) also contribute to arterial stiffening by cross-linking with the less elastic collagen fibers [27]. Functional arterial stiffening can be linked to an increase in VSMC tone, hypertension, and endothelial dysfunction [28]. Both sympathetic activity and endothelial derived factors influence VSMC tone and contributes to the progression of stiffness in MetS individuals [28]. The endothelium is a single layer of cells that line the arteries and release the potent vasodilator, NO [29]. NO suppresses VSMC contraction and prevents adhesion of platelets and monocytes that lead an increase in CVD risk [30]. It has been show that MetS patients suffer from endothelial dysfunction, mainly due to the decrease in NO bioavailability [31]. This decrease in vasodilator capacity and migration protection can increase wave pressures and wall hypertrophy, increasing arterial stiffness.

In a stiffened aretery, an increase in the pressure wave places more tension on the microcirculation, and early reflection, due to decreased arterial elasticity, increases the workload on the LV [5]. This failure to cushion pulsations results in an increased risk for renal disease and stroke [32, 33]. The increased workload on the heart can lead to the development of an enlarged heart, left ventricle

hypertrophy (LVH), increasing the risk for heart failure and myocardial infarction [5]. Finally, in a healthy artery the return of the pulse wave during diastole boosts perfusion to the coronary arteries. In a stiffened artery the earlier return can decrease coronary perfusion, increasing the risk of myocardial ischemia [6].

Exercise training is a well-established therapeutic intervention, and effective physiological stimulus that reduces CV related events and the possibility of developing CVD [34-36]. Regular aerobic training is associated with less stiffening of the large arteries in healthy adults, compared to their sedentary counterparts [37]. More importantly, regular aerobic exercise has been shown to reverse some of the arterial stiffening imposed in previously sedentary, middle-aged and older men [25]. Aerobic training has also improved arterial elasticity in type II diabetics and hypertensive individuals; along with the improvement in endothelial function and reduced IMT [38, 39]. Studies have also shown that MetS patients who implemented an exercise-training regime improved endothelial function [16, 40]. However, it is not known whether continuous, aerobic exercise training can attenuate arterial stiffness or reverse premature arterial thickening in MetS individuals.

Several mechanisms may be responsible for the cardioprotective effects of regular aerobic exercise on stiffness of large arteries. These may include both systemic and/or local effects on the arterial wall, directly influencing wall thickness, endothelial function, and elasticity [41]. Continuous exercise increases localized shear stress, increasing NO bioavailability, improving endothelium-dependent vasodilation [42]. Localized shear stress can also decrease

vasoconstrictors, preventing VSMC tone and wall thickening [43]. Other studies have shown a systemic effect on endothelial function through an exercise regime of the lower limb improving vasodilation capacity in the upper limb [44]. Data on whether exercise training prevents, or reverses premature carotid IMT, are equivocal. One study showed that after 3 months of high-intensity aerobic exercise, carotid IMT was not decreased in a healthy, previously sedentary population [45]. However, another study of the same nature, looking at conduit artery IMT, showed a decrease in thickness after an 8 week intervention [2].

A primary public health concern is to understand the mechanisms by which the MetS is associated with CVD, and to establish appropriate prevention and intervention strategies. One possibility may be due to the increase in arterial stiffening observed in this population. Our goal is to examine the impact of an aerobic exercise intervention on arterial stiffness and wall thickness in the MetS population.

# **Chapter 2**

## **Review of Literature**

## **2.1. The Metabolic Syndrome and Cardiovascular Disease**

CVD is the leading cause of morbidity and mortality in modern societies [46]. Over 82 million, 1 in 3, Americans has some form of CVD [15]. Several conditions increase a person's risk of developing CVD. These factors include a family history of premature coronary artery disease (CAD), hypertension, hyperlipidemia, high cholesterol, insulin resistance/hyperglycemia, smoking, as well as aging [47, 48]. Certain CVD risk factors occur together and are not independent of one another in their shared responsiveness to lifestyle modifications or increased physical activity [49, 50]. The MetS is a clustering of risk factors that lead to a high risk for the development of CAD, type II diabetes and CVD [51-54]. Individuals with the MetS are 3 times more likely to die of heart disease than the healthy population [16]. It is estimated that 56 million (24%) Americans, over the age of 18, have MetS and 45% of people over the age of 50 have MetS [17, 52]. The MetS components include; abdominal obesity, low HDL cholesterol, elevated triglycerides, hyperglycemia, and hypertension [55]. Arterial dysfunction, due to arterial stiffening, is recognized as a surrogate end point for CVD and a number of studies point to stiffness predicting the risk of future fatal and nonfatal CV events [6, 7, 27]. The MetS has more advanced stiffening, when compared to their healthy counterparts [19, 22]. Carotid IMT, a non-invasive measurement of arterial wall thickness, was shown to increase 2-to-3 fold between 20 and 90 years of age in a population of 137 healthy, sedentary men [45]. A meta-analysis found that a 0.1 mm increase in carotid IMT is associated with an 18% increased risk for stroke and 15% for myocardial infarction [56].

Several studies have found that physically active men and women have a lower incidence of CVD compared with sedentary peers [36, 57, 58]. Regular aerobic exercise is associated with enhanced arterial compliance and greater endothelial function, especially in healthy middle-aged and older adults, that may be responsible for much of the CV protective role due to training [59].

### **2.1.1. Definitions of the Metabolic Syndrome**

Identifying those with increased metabolic risk components is important, because it categorizes people who are at a high risk of developing CVD and type II diabetes, such as the Framingham risk score and a diabetes prediction model [55]. The term identifies a specific subgroup of individuals with a shared pathophysiology, easily used between clinicians, recognizing an underlying biological process [55]. There are four commonly used definitions of the MetS, summarized in table 2.1 below. [60]. The World Health Organization (WHO) was the first definition, released in 1998 [61]. It tied together the key components of insulin resistance, obesity, dyslipidemia, and hypertension. It stated that insulin resistance must be present and even if all the other criteria were met, the individual would not have the MetS [61]. The European Group for the Study of Insulin Resistance (EGIR) released a modified definition in 1999 that defined insulin resistance levels, and stated that resistance, plus two other risk factors must be present [62]. The modification also excluded those with diabetes [55]. In 2005 International Diabetes Foundation (IDF) published new criteria for MetS. The IDF does not require insulin resistance, but does stress the importance of

weight distributions, recognizing central obesity as an important factor. The IDF definition is often criticized for its emphasis on obesity, rather than insulin resistance [63].

The definition used during this study, according to the National Cholesterol Education Program: Adult Treatment Panel III (NCEP ATP III), is defined as the presence of three or more of the following traits: abdominal obesity, low HDL cholesterol, elevated triglycerides, hyperglycemia, and elevated BP [18]. It is one of the most widely used definitions because it incorporates measurements and laboratory results that are readily available to physicians and clinical researchers. It isn't biased, because it does not require any one of the specific criterion to be met towards an underlying cause of MetS, just that 3 of the 5 are present [55].

Table 2.1. Definitions of the metabolic syndrome

| <b>Criteria</b>        | <b>NCEP ATP III</b>                               | <b>WHO</b>                                 | <b>EGIR</b>                              | <b>IDF</b>  |
|------------------------|---|--|--|---|
| <b>Requirements</b>    | 3 of 5 criteria below                             | Diabetes or IR, plus 2 of 5 criteria below | High Insulin, plus 2 of 4 criteria below | Obesity, plus 2 of 4 criteria below               |
| <b>Obesity</b>         | Waist >102 cm (M), >88 cm (W)                     | Waist/hip ratio >0.9 cm (M), >80 cm (W)    | Waist >94 cm (M), >80 cm (F)             | Obesity required                                  |
| <b>Hyperglycemia</b>   | Glucose >100 mg/dl                                | IR required                                | IR required                              | Glucose >100 mg/dl                                |
| <b>Dyslipidemia</b>    | TG >150 mg/dl                                     | TG >150 mg/dl                              | TG >177 mg/dl                            | TG >150 mg/dl or RX                               |
| <b>HDL Cholesterol</b> | HDL-C <40 mg/dl (M), <50 mg/dl (W)                | HDL-C <35 mg/dl (M), <39 mg/dl (F)         | HDL-C <39 mg/dl                          | HDL-C <40 mg/dl (M), <50 mg/dl (F) or RX          |
| <b>Hypertension</b>    | >130 mmHg systolic or 85 mm Hg diastolic BP or RX | >140/90 mmHg BP                            | >140/90 mmHg or RX                       | >130 mmHg systolic or >85 mmHg diastolic BP or RX |

\* IR – Insulin Resistance, TG – Triglycerides, RX – Medicinal Treatment

### **2.1.2. Normal Arterial Function**

Blood flows continuously, evenly, and uninterruptedly through the arteries of the body, whereas the heart's action as a muscle pump is intermittent [6]. The central arteries play two important roles in its interaction with the LV, that of a conduit and a cushion. The first function is to deliver blood from the LV to the smallest arteries of organs and tissues, according to their needs. The second is to cushion the pulsations generated by the heart so that blood flow is continuous [5]. The large central arteries, particularly the aorta where elastin material is greatest, are very elastic and act as a reservoir during systole, storing part of the ejected blood, forcing it out into the peripheral arteries during diastole. A.V. Hill stated, "The amount of energy which the heart has to expend per beat other things being equal, varies with the elasticity of the arterial system" [6]. A healthy, compliant aorta results in an efficient heart and arterial interaction, minimizing any pressure increases imposed on the heart that would cause an increase in the workload.

Following the systolic ejection of blood on the aortic wall, a pressure wave develops and is rapidly propagated from the LV down the arterial system [26]. When the forward wave reaches high-resistant arterioles a backward wave occurs as a consequence of wave reflection. The reflected wave summates with the forward traveling wave, creating a central pressure waveform. A healthy arterial system is most efficient when the proximal aorta is elastic, resulting in a wave reflection that returns during diastole, optimizing perfusion of the coronary arteries [6].



### **2.1.3. Arterial Stiffness Defined**

The structure and function of the central arteries, particularly the aorta, change throughout the lifetime of humans. This arterial bed is elastic and compliant in a healthy individual, storing blood ejected during contraction of the heart and releasing it to the periphery during diastole. As a person ages the central arteries become less compliant [5]. Arterial stiffening, known as arteriosclerosis, is defined by lumen enlargement with wall thickening (remodeling) and a reduction of elastic properties (stiffening) [10]. In a healthy, young individual the PWV is approximately 3 to 5 m/s at rest, increasing 2-to-3 fold with age [37].

Increased stiffness predicts the development of CVD and is associated with common diseases of aging, including stroke and renal disease [64-67]. Arterial remodeling contributes to most age-associated CV disorders including; systolic hypertension, increased cPP, LVH, diastolic dysfunction, stroke, atherosclerotic coronary and peripheral disease, congestive heart failure, and myocardial infarction [27, 68]. Arterial aging is believed to originate from alterations in cross-linking of extracellular matrix components, elastic network degradation, and calcification of elastic fibers [10]. Stiffening of the large arteries reduces capacitance, contributing to the widening of cPP and the increased prevalence of systolic hypertension with age [47]. The chronic rise in central pressures increases the risk of carotid thickening, via hyperplasia and hypertrophy of VMSC and increased collagen deposition [69], further contributing to arterial stiffening. Enlargement of the large arteries with age, and increased pressures, is generally

attributed to the fracture of load-bearing elastin fibers. The arterial wall remodels itself in response to long-term changes in increased pulsatile flow, such as the luminal diameter expands to maintain a constant, pre-determined, level of shear stress [70]. A previous study found a strong relationship between increased cPPs and both carotid wall thickness and lumen enlargement [71].

## **2.2. Arterial Stiffness and Assessment Methods**

Central carotid-femoral PWV (PWVcf) represents the time taken by the pulse to travel between two points of known distance, and is a direct measure of arterial stiffness [10]. Arterial stiffening is associated with numerous CVD risk factors [47, 48] leading many to believe that increased stiffness may be a surrogate marker for advanced atherosclerotic disease. However, prior studies have suggested that aortic stiffening can occur in the absence of atherosclerosis [25, 37]. A study conducted in China, where atherosclerotic disease is almost nonexistent, allowed the independent investigation of the effects of age and BP on PWV, without any or less significant contribution from atherosclerosis [25]. Both SBP and PP increased with age and were significantly related to PWV [25]. PWV was not related to serum cholesterol, which tended to be lower at all ages compared to that of Western populations. This indicates that aging, not atherosclerosis, is the dominant factor associated with reduced arterial compliance.

DBP, mean arterial pressure, and peripheral BPs were considered to be a better guide of disease severity, but the paradigm has shifted over the past few decades, and many studies have shown cPP is increased with advancing age

and is a stronger predictor of CVD risk [24, 47]. Central pressures are a representation of strain imposed directly on the heart [24]. In a population-based cohort of the Framingham Heart Study, Franklin et al. [72] demonstrated during a 20-year follow-up period, that cPP rose steeply after the age of 50. In addition to PWV as a measure of arterial stiffness, simultaneous evaluation of the cPP provides an indirect assessment of stiffness [73]. cPP is the consequence of intermittent ventricular ejection from the heart and its main role is to minimize pulsatility in the central arteries [47]. The determinants of cPP are the cushioning capacity of arteries and the timing and intensity of wave reflections [26]. During pressure increases the elastic properties of the arterial media reach a point where less distensibility occurs [74]. At low pressure elastin fibers dominate the mechanical behavior and the arterial wall is very extensible. Whereas at higher pressure the tension is predominantly absorbed by the more rigid collagen fibers, reducing compliance [26]. Measurements of PWV should be corrected for the acute response of a rise in pressure.

Applanation tonometry provides a non-invasive measure of central pulse waveform analysis (PWA), producing an Alx measurement [24]. The radial pressure wave is recorded and a validated generalized transfer function is applied to generate the corresponding central pressure waveform. Alx is a measure of systemic arterial stiffness, representing the percentage of cPP that is constituted by the augmentation pressure added to the systolic pressure peak, based on the reflected wave [24]. In a stiffened artery the quicker moving wave returns during the systolic ejection period, increasing the cardiac workload, which

may overtime lead to LVH. A hypertrophied heart requires more oxygen and contracts more slowly, decreasing the duration of diastole, linking LVH to myocardial ischemia, angina, atrial fibrillation, and heart failure [75]. This decrease in diastolic period, along with a slower HR and earlier reflected wave, adds to the workload mentioned above. SBP is amplified by the early reflected wave and diastolic augmentation is reduced, decreasing DBP, leading to an elevation in cPP [74]. This shows cPP not only can lead to an increase in arterial stiffness, mainly acutely at higher pressures as the workload is pushed to the more rigid collagenous fibers, but also that a stiffened artery contributes to hypertension through a chronic increase in cPP.

Ultrasound examination of the carotid artery IMT has traditionally been used to evaluate the presence of atherosclerosis [76]. More recently it has emerged as one of the most popular methods in detecting non-obstructive plaque and determining early arterial wall remodeling [76, 77]. Several studies have shown that increased IMT is a predictor of CV events [56, 76, 78]. IMT is increased in a number of CV risk factors including: diabetes, hyperglycemia, hypertension, obesity, and MetS individuals [11, 27, 79, 80]. More importantly, IMT occurs with aging, even in populations without incidence of atherosclerosis [81]. A thickened carotid artery is relevant in the discussion of arterial stiffening because of the changes that occur in arterial wall composition in a stiffened artery. Increased stiffness contributes to a greater rise in cPP [71]. The rise in central pressure increases the risk of carotid thickening, in response to insults via hyperplasia and hypertrophy of VMSC and increased collagen deposition [69].

## **2.3. Arterial Stiffness and Possible Mechanisms**

There are several structural and functional arterial changes that occur which influence arterial stiffening. Hypertension, one of the MetS criteria, accelerates arterial stiffening through both structural and functional mechanisms [26]. The increased pulsations caused by elevated BP places extra stress on the weight-bearing elastic lamellae, resulting in central artery stiffening [82]. Acquiring additional knowledge about the structural and functional deviations of the arteries will provide an opportunity to develop therapeutic interventions with a specific target of action.

### **2.3.1. Structural Arterial Stiffening**

Arteries stiffen with age principally because of elastin fiber fracture and the resultant increase in collagen/elastin ratio. In a normal functioning artery the amount of elastin material is predominately located in the central arteries, where it plays the key role of cushioning blood flow and pulse waves from the LV. This majority is reversed in muscular arteries, where there is a far greater amount of collagen [26]. As a result, carotid-radial (PWV<sub>cr</sub>) is greater at a younger vs. older age and doesn't experience the increase in stiffening witnessed in the central arteries [6]. The proximal, elastic arteries and aorta dilate approximately 10% during ventricular contraction, as opposed to only 2% to 3% for the peripheral, muscular arteries [5]. Natural rubber with repetitive stretch of 10% will break after 1 billion cycles, which corresponds to 30 years of a human life [6]. Therefore, an individual who lives to an age of 90 has experienced close to 3 billion

contractions of the heart, and the same number of pressure loads imposed on the proximal arteries. The PWVcf reaches, and often exceeds, the value observed in muscular arteries with aging, and is accelerated in the MetS [21]. Increased central pressure acutely transfers the stress of the pulse wave from elastin material to less distensible collagenous fibers in the arterial wall, and chronically increases collagen production and AGE linkage to the extracellular matrix material. Many would argue then that stiffening is a natural consequence of aging and that the famous John Hopkins physician, William Osler, is correct in stating that “man is as old as his arteries [5].”

In addition to the elastin fiber fracturing, and the outward wall remodeling observed in MetS patients, increased collagen concentration and cross-linking, VSMC hypertrophy, and increased growth factors are key mechanisms leading to structural changes in the intima-media layer that cause stiffening [27]. The amount of collagen is determined at a very young developmental stage and experiences very low turnover [26]. However, as presented earlier, repetitive pressure strains imposed on the arterial wall results in a proximal aortic collagen-to-elastin ratio remodeling towards that of the more distal arteries. Several neurohumoral factors, particularly angiotensin II and aldosterone systems, modulate collagen accumulation. It has been shown in cell cultures, angiotensin II stimulates the production of various types of collagen, VSMC hypertrophy, and growth fibers [28]. Also, AGEs (proteins or lipids that become glycosylated after exposure to sugar) contribute to arterial stiffening by forming irreversible cross-links between slow-turnover proteins such as collagen, and compromise

endothelial function by decreasing NO and increasing the generation of oxidants [3, 4]. AGEs activate metalloproteinases (MMPs), initiating inflammatory responses that can increase arterial stiffness, further contributing to endothelial dysfunction and atherosclerotic disease promotion [83]. MMPs are enzyme producing proteins which control extracellular matrix components and when over expressed can lead to an increase in collagen and stiffening [84].

### **2.3.2. Functional Arterial Stiffening**

Functional changes that have a role in arterial stiffness are increased VSMC tone (increased sympathetic nervous activity), high BP, and endothelial dysfunction [23, 85]. VSMCs don't represent a homogenous population, they may have different contractile, proliferative, and apoptotic behavior [4]. The expression of each of the phenotypes depends not only on age, but also on the location in the arterial system and pathological conditions [26]. Changes in VSMC tone, causing arterial constriction or dilation, can occur directly or through signals from the endothelial cells, influenced by shear mechanical stress, as well as paracrine mediators such as angiotensin II, endothelin, and NO [26]. Increased sympathetic nervous system activity, present in aging and MetS patients, influences VSMC tone and the progression of arterial stiffness [86].

The endothelium, made up of a single layer of cells that line the arteries, provide a critical barrier of protection between the blood and tissues [30]. The vasodilator NO, is released from the endothelium and limits clotting and inflammation, suppresses VSMC contraction, and prevents adhesion of platelets and

monocytes that all contribute to arterial remodeling [29]. Traditional risk factors of endothelial dysfunction are hypertension, cigarette smoking, diabetes, age, hypercholesterolemia, and CAD; owing to decreased bioavailability of NO, mainly contributable to evidence of oxidative stress [87-92]. Recent studies have shown that this loss of endothelium-dependent vasodilation plays a key role in arterial stiffening [1, 93]. A proposed central event in the MetS is a decrease in the amount of bioavailability of NO from endothelial cells, increasing VSMC tone [23]. The increase in VSMC tone can alter the distribution of stresses between the elastic and collagenous fibers of the arterial wall and thus alter stiffness. Evidence also indicates that arterial stiffness itself may influence endothelial dysfunction, and NO release [94, 95]. A chronic increase in PP can potentially alter the eNOS responses. The wall thickening that can occur in a stiffened artery poses a barrier to NO diffusion from endothelial cells, which may cause endothelial dysfunction [96]. Flow-mediated dilation (FMD) is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress, and is the method used in our study to observe functional stiffening.

## **2.4. Cardiac Consequences of Arterial Stiffness**

In a stiffened aorta the reflected pressure wave returns earlier in the cardiac cycle, increasing the workload of the heart, resulting in LVH [5]. A hypertrophied heart requires more oxygen and becomes predisposed to ventricular systolic or diastolic heart failure, left atrial strain and enlargement, and atrial fibrillation [24].



Further, the hypertrophied heart contracts more slowly, the duration of systole lengthens, decreasing the length of diastole [5]. The coronary arteries are mostly perfused during diastole, and in a stiffened vessel the earlier return of the reflective wave during systole impairs perfusion, increasing the risk of myocardial ischemia [6]. Increased large artery stiffness most likely explains the fall in DBP, commonly observed after the age of 60 years or accelerated in MetS patients. The decline DBP is probably the result, rather than the cause of stiffening [97].

Arterial stiffening also increases the risk for stroke and renal malfunction. The microcirculation is compromised mainly of smaller, resistance arteries that act primarily as conduits, not playing a role in cushioning [5]. As the central arteries lose elasticity with age and disease, the failure to cushion pulsations leads to an increase in pressure wave transfer to the peripheral organs, mainly the brain and kidney [98]. Severe lesions in the microcirculation of the brain and kidney are observed in older persons that include damage to the endothelium with thrombosis and inflammation of the media [32]. Therapeutic interventions that reduce stiffness improve small arterial function and decrease both cerebral and renal dysfunction, linking stiffness to microcirculation disease [33, 99, 100].

## **2.5. Metabolic Syndrome and Arterial Stiffness**

As a result of CVD risk factor clustering, MetS patients often have arteries that have stiffened beyond their chronological age [19, 20, 22, 101]. It places this population at an increased risk of multiple diseases [102]. An important MetS risk factor, high BP, can have a drastic effect on arterial stiffening [21, 103]. This

repetitive strain in MetS patients is greater than that of a disease free individual, causing greater elastin fiber fracture and less cushioning capability of the arteries [6]. Other risk factors associated with the MetS, hyperlipidemia and low HDL cholesterol, influence the functional capacity of the endothelium, which increase stiffness [104].

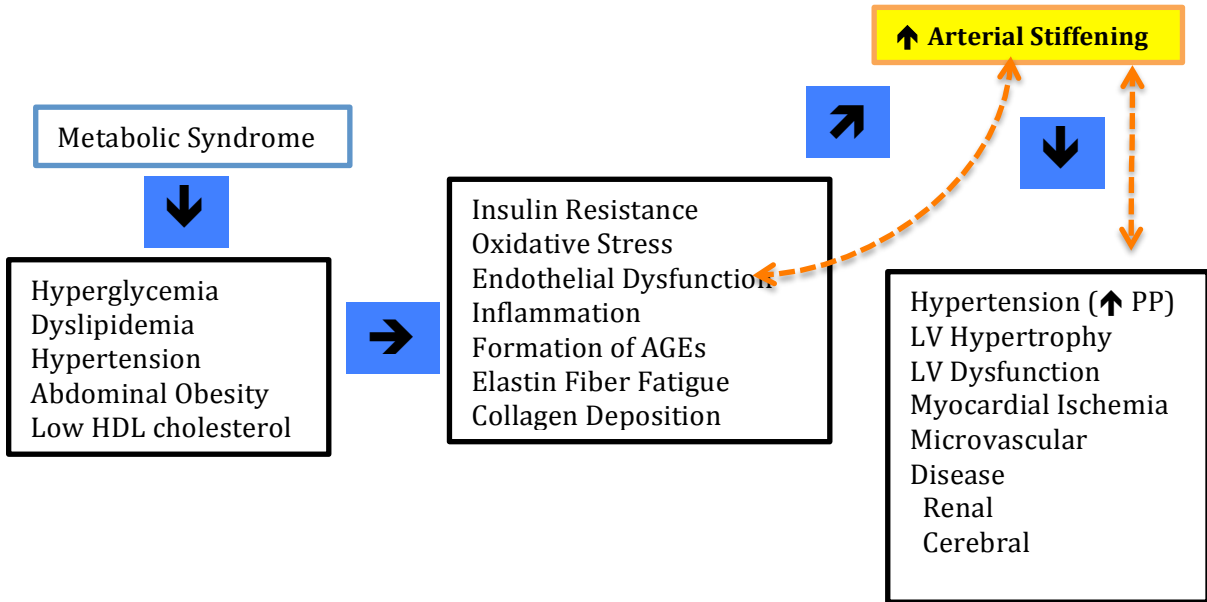


Figure 2.1. Summary of MetS components & CVD risk

## 2.6. Aerobic Exercise and Arterial Stiffness

Hippocrates taught, “positive health requires a knowledge of man’s primary constitution...There must also be exercise of which the effects must likewise be known...If there is any deficiency in exercise the body will fall sick” [34]. The teachings were based on inspiration and philosophy, without scientific evidence. However, the development and growth of physical activity research has proven, and continues to produce, various mechanisms of disease protection. Exercise

training is a well-established therapeutic intervention and effective physiological stimulus that reduces CV events and the possibility of developing CVD [34-36, 105]. Exercise training has been shown to improve arterial function in healthy adults (previously sedentary), and hypertensive individuals, which relates to a decreased risk of CVD [2, 41, 44]. Arterial stiffness imposes a threat on arterial wall remodeling, increasing the incidence of CV risk factors, and influences the release of endothelial derived factors, affecting oxidative stress and inflammation, which has been shown to be attenuated with exercise [47]. Regular aerobic exercise has been shown to attenuate arterial stiffening observed in previously sedentary, healthy middle-aged and older men [50, 59]. Exercise also reduces artery wall thickness in other populations, associated with reduced rates of hypertension and other CV disease states [2, 45]. An important cardiac and arterial protective effect of exercise training may be explained by the effect of acutely elevated pulsations imposed on the arteries [106].

### **2.6.1. Aerobic Exercise and Arterial Stiffening In Other Populations**

The protective effect against arterial stiffness has been shown to be related to cardiorespiratory fitness [37]. Arterial stiffening was shown to be inversely related to aerobic capacity in healthy, endurance-trained and sedentary populations [25]. More importantly, in previously sedentary, healthy middle-aged and older men who underwent 3 months of aerobic exercise training, carotid artery compliance returned to levels observed in exercise-trained adults of the same age [107]. Multiple studies have shown that exercise training in younger endurance-trained

individuals and older, previously sedentary adults, improved endothelial function and reduced IMT [2, 43, 106, 108, 109]. The Framingham Heart study showed that untreated hypertension may contribute to increased arterial stiffening and thus perpetuate a vicious cycle of accelerated hypertension and further increases in large artery stiffness [82]. A 3 week aerobic exercise protocol decreased arterial stiffness in both the carotid and femoral arteries in hypertensive type II diabetics [110]. The reduction occurred without any significant improvements in aerobic fitness, weight, body mass index (BMI), or BP.

### **2.6.2. Aerobic Exercise and Arterial Stiffening In the Metabolic Syndrome**

Previous studies have shown that aerobic exercise and strength training improves endothelial function and capacity in MetS patients after 12 to 16 weeks [16, 40]. Whereas short-term (10 days) training had no effect on endothelial function or PWV in MetS individuals [49]. No difference was observed in FMD or PWV between MetS patients and healthy controls who trained [49]. However, an inverse relationship was identified between cardiorespiratory fitness and central stiffness in men with the MetS [111]. Therefore, it is believed that a longer duration of continuous, aerobic exercise will decrease arterial stiffness and carotid IMT in MetS individuals. No study has shown the effect of chronic aerobic exercise on carotid IMT or stiffness in the MetS. Previous studies have shown an attenuation of both after an 8-week intervention in other populations [38, 112].

## **2.7. Possible Mechanisms for Reduced Arterial Stiffness**

The mechanisms underlying the effects of regular aerobic exercise on large elastic artery stiffness are largely unknown, but are likely due to functional alterations, possibly due to improved endothelial function as already shown [16, 40], or changes to the composition of the arterial wall [2]. The present study was not designed specifically to determine the mechanisms underlying the effects of regular exercise on arterial stiffness and remodeling. However, we hope that our data may provide insight into this question.

### **2.7.1. Aerobic Exercise and Endothelial Function**

The cardioprotective effects of exercise training may be explained in part by the direct effects of repeated exercise bouts on the arteries, influencing endothelium-dependent vasodilation [105]. In one study subjects were asked to perform a bilateral handgrip-training program, where one arm was occluded with a blood pressure cuff to simulate a decrease in blood flow and shear stress. The same exercises were performed in each arm, but vasodilator function, measured by FMD, was only enhanced in the limb exposed to increases in shear stress during the training bouts [106]. This confirmed that pulsatile flow, through shear stress, provides a physiological stimulus to NO production, promoting a decrease in inflammation and VSMC tone. Changes in shear stress also decrease the expression of vasoconstrictors, endothelin-1 and VSMC adhesion molecules, exhibiting an anti-atherogenic effect [106].

However, it was also shown that lower limb exercise training improved endothelial function in the upper limb arteries [42]. This suggests, in addition to localized action, exercise training induces generalized systemic effects on endothelial function, either through an increase in circulating metabolic stimulus, anti-inflammatory response (antioxidant enzymes), or systemic hemodynamic changes [2, 42]. The capacity for NO vasodilator activity to increase in non-exercised limbs indicates that acute exercise training is associated with a sustained and systemic favorable modification of arterial stiffness, at least in the short-term. Thus, acute increases in BP, and the consequent cyclic change in transmural pressure across the arterial wall, may contribute to functional adaptations [113]. In summary, NO activity in the resting arteries is enhanced through exercise-mediated, hemodynamic modulation in active tissues, influencing VSMC tone and altering the distribution of stresses between the elastic and collagenous fibers of the vessel wall [23].

### **2.7.2. Aerobic Exercise and Oxidative Stress**

Oxidative stress can influence endothelial dysfunction by suppressing the bioavailability of NO [30]. However, in low concentrations, free radicals are thought to act as mediators and modulators of cell signaling and are important in exercise-induced vasodilation. A recent study of human coronary resistance arteries showed that non-muscle mitochondrial respiration and oxidative stress, through an increase in exercise-related shear stress and the consequent release of free radicals, is necessary for flow-mediated dilation to occur [114]. Oxidative

stress is determined by the balance between pro- and antioxidant forces, and is likely a crucial aspect of life [115]. Richardson et al [116] implicated an exercise-induced reliance upon pro-oxidant-stimulated vasodilation during an acute bout of exercise in young healthy men. They also showed that an oral antioxidant cocktail effectively reduced free radicals, disrupting the balance between pro- and antioxidant forces, decreasing the exercise-induced endothelial-dependent vasodilation [116]. In contrast, at high levels the superoxide ( $O_2^-$ ) and other free radicals are believed to inactivate NO, contributing to the progression of endothelial dysfunction and resultant increase in arterial stiffening [115].

The increase in oxidative stress and inflammatory action observed in MetS individuals, can be attenuated with aerobic exercise training [117]. One study showed that aerobic exercise inhibited NADPH oxidase activity (increases reactive oxygen species) and up-regulated the antioxidant enzyme, superoxide dismutase, improving conduit artery dilation in old sedentary mice by increasing NO bioavailability [118]. Further, it was demonstrated that older men who exercise regularly do not demonstrate arterial endothelial oxidative stress, and oxidative circulating factors did not differ with young controls [119].

### **2.7.3. Aerobic Exercise and Arterial Wall Remodeling**

Several studies have been performed looking at the effect of exercise training on wall thickness in different arterial locations, and have proven to be inconsistent in their findings. Thijssen et al. [2] showed that exercise training in healthy, previously sedentary, young men led to a decrease in conduit wall thickness due

to systemic, rather than localized stimuli. However, several previous studies showed that training does not impact carotid artery thickness or diameter [45, 120]. Tanaka et al. [45] performed a cross-sectional study inclusive of young and old people, comparing healthy, sedentary individuals to healthy, endurance trained athletes. They concluded that regular vigorous endurance exercise did not attenuate the age-associated increase in IMT [45]. An additional healthy, sedentary group was recruited to perform a 3-month aerobic exercise intervention and found that carotid IMT did not decrease after exercise [45]. One theory may be that SBP or cPP did not decrease following the exercise, which has been shown to have a major influence on arterial remodeling [10]. The length of exercise duration, extent of arterial thickening, and most importantly, the population observed must be considered. Only a healthy cohort, with no evidence of chronic diseases, has been studied. It is possible that regular aerobic exercise could have a beneficial effect on carotid IMT in MetS individuals with increased CV risk factors.

## **2.8. Future Implications for Cardiovascular Disease**

The number of Americans over 60 will nearly double, from 57 million people in 2000 to over 92 million in 2030, an increase from 18 to 25 percent of the population [121]. If the current trends hold true in the future, a similar increase in the prevalence of the MetS will most certainly occur. Thus, the incidence and prevalence of age-associated, arterial disease will increase dramatically. There is individual variability in arterial dysfunction and stiffening often associated with



advancing age [20]. This suggests that several physiological and lifestyle factors have a great influence on arterial aging. Habitual aerobic exercise may be the single most important modulatory influence [46]. Many of the current therapeutics, including antihypertensive drug therapy and exercise training programs, were mainly designed to reduce peripheral resistance and are not adequately designed to alter the pathological process of central arterial stiffening. The goal of our research is to determine whether aerobic exercise training can attenuate arterial stiffening and wall thickness in MetS individuals.

# **Chapter 3**

## **Methods**

## **3.1. Ethics and Medical Screening**

### **3.1.1. Study Eligibility Screening**

Ethical approval was obtained from the West Virginia University ethics committee (IRB# H-22147). All subjects received verbal and written explanation of the procedures involved and signed consent forms.

Preliminary screening to determine study eligibility of the subjects was accomplished by completing a medical health questionnaire, which ascertained any past history of CV conditions and any other medical conditions that may conflict with testing. A twelve-lead electrocardiography (ECG) (Nihon Kohden Cardiofax GEM) was performed in a sitting position to rule out any cardiac abnormalities that would prevent exercise capability. Medical exclusion criteria for entry into the studies included those diagnosed as having CAD (angiography, stress testing, and other physician determined means), diabetes mellitus, myocardial infarction, or stroke. Individuals were selected from a large pool of people that responded to local advertisements asking for volunteers.

### **3.1.2. Risk Factor Assessment**

Waist Circumference, in centimeters (cm), was measured at the level of the umbilicus using a tape measure. Resting BP levels were taken as the average of two or three measurements, recorded at least 2 minutes apart, in the right arm with an automated pressure monitor (Omron Healthcare Inc.). Participants rested for at least 15 minutes before BP was measured. Weight was measured by a

mechanical balance (Detecto®), height by a stadiometer (Detecto®), and BMI was calculated in units of kg/m<sup>2</sup>. Body fat percentage was obtained through the assessment of skinfold thickness, measured at 7 sites (triceps, pectoral, subscapula, midaxilla, abdomen, suprailiac, quadriceps) on the body. The measurements were then entered into a formula (ExRX.net Body Composition Calculator), converting the values into an estimate of each individual's percent of body fat, according to a person's age and gender. Blood samples were obtained after an overnight fast. Blood plasma glucose, HDL cholesterol, and triglycerides were analyzed at the hospital Clinical Medicine Laboratory, enzymatically using standard procedures.

### **3.2. Experimental Design**

The purpose of this study was to examine the impact of 8 weeks of aerobic exercise training on arterial stiffness and wall thickness in individuals with the MetS. The interventional study design includes 2 groups; the first consisting of MetS individuals performing exercise and a group of MetS individuals who maintained their normal lifestyle. The presence of the MetS is defined according to the NCEP ATP III definition [17]. Each subject underwent two evaluation sessions, before and after the 8-week intervention. Participants reported to the laboratory after an overnight fast, abstaining from medications, caffeine, and vitamins the morning of the tests. All study sessions were performed with the subject supine, and took place between 7:00 and 10:00 AM to avoid circadian rhythm bias. Applanation tonometry of the radial artery was performed, producing

the cPP waveform and central pressure measurements [122]. Wave reflection, an integral part of the waveform, was assessed through AIx. Arterial stiffness was assessed as PWV using the Sphygmocor (AtCor Medical Pty. Ltd., Sydney, Australia). Carotid IMT, used to evaluate arterial remodeling, was measured from the images derived from an ultrasound machine (GE Vividi). Finally, FMD, a noninvasive measurement, was employed to observed endothelial function [31].

### **3.3. Subjects**

#### **3.3.1. Metabolic Syndrome Individuals**

Twenty-one individuals (9 men and 12 women, average age  $47 \pm 3$  years) with the MetS were included in this study. Participants were split into to either the aerobic exercise training group (n=11) or a sedentary group (n=8), which maintained their normal lifestyle and activity level during the duration of the study. Two participants did not complete the exercise training protocol, and were therefore included only in the cross-sectional analysis. MetS was defined according to the NCEP ATP III [17], by the presence of 3 or more of the following traits:

- Abdominal obesity (>102 cm in men and >88 cm in women)
- Low HDL cholesterol (<40 mg/dL in men and <50 mg/dL in women)
- Hypertriglyceridemia (>150 mg/dL)
- Hyperglycemia (>100 mg/dL)
- High blood pressure (>130/85 mm Hg) or hypertensive medications

### **3.3.2. Healthy Control Individuals**

Fifteen healthy, control subjects (6 men and 9 women, aged  $41 \pm 3$  years) were enrolled in the study. None were diagnosed with CVD, hypertension, or abdominal obesity.

All potential subjects were excluded if they suffered from diabetes mellitus, chronic pulmonary disease, symptoms of unstable angina, aortic stenosis, anemia, cancer requiring treatment within the last 2 months, or orthopedic or neurologic disability that would limit the ability to exercise. Individuals who have had a myocardial infarction, stroke, or coronary revascularization were also excluded. Subjects who participated in regular exercise (running, cycling, swimming, weight lifting, etc.) defined as greater than 30 minutes at least 3 times a week were excluded.

### **3.4. Experimental Procedures and Training Protocol**

Exercise training was performed over an 8-week period with subjects visiting the on-site gym, the Human Performance Laboratory. Each session consisted of 60 minutes of aerobic exercise training, 30 minutes on a treadmill and 30 minutes on a bicycle. Initially, subjects exercised at 60% of their individually determined heart rate (HR) reserve, obtained during the measurement of peak aerobic capacity ( $VO_{2peak}$ ). Every two weeks the exercise intensity increased from 60% to 70%, 80%, and finally 85% of their HR reserve. Adherence to the exercise prescription was documented through the use of Polar HR monitors and physical

activity logs. All measurements were performed at least 48 hours following the last training session. Across the exercise-training period, there was 90% adherence to the training sessions. Subjects not participating in the training were instructed to maintain their normal daily lifestyle and activity level.

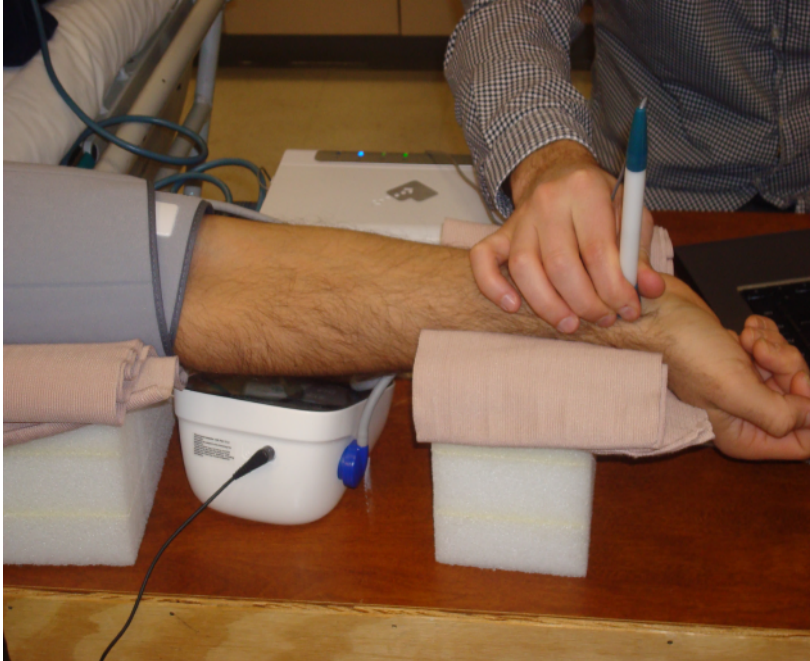
Participants performed an incremental exercise stress test on a cycle ergometer, using the Bruce protocol to obtain maximal HR. The ergometer was placed at a 45-degree angle for acquisition of arterial and cardiac parameters during the stress test. Expired gases were collected using a one-way valve and analyzed using a metabolic cart (ParvoMedics TrueOne 2400).  $VO_2$ peak was defined as the highest recorded value during the test. This is in contrast to maximal oxygen uptake, which occurs when oxygen consumption plateaus and exercise continues, even though the maximum is achieved. HR was recorded halfway through each 3-minute stage and at the end of each stage using 12-lead ECG. BP was measured by an investigator using a manual sphygmomanometer at rest, during each minute and a half stage, and at maximal exercise. Exercise was stopped for any of the following reasons: a 20 rating of perceived exertion, if the participant achieved age-predicted maximal HR, or if the individual was too fatigued to maintain a cadence of 50 revolutions per minute.

## **3.5. Experimental Measures**

### **3.5.1. Pulse Wave Analysis**

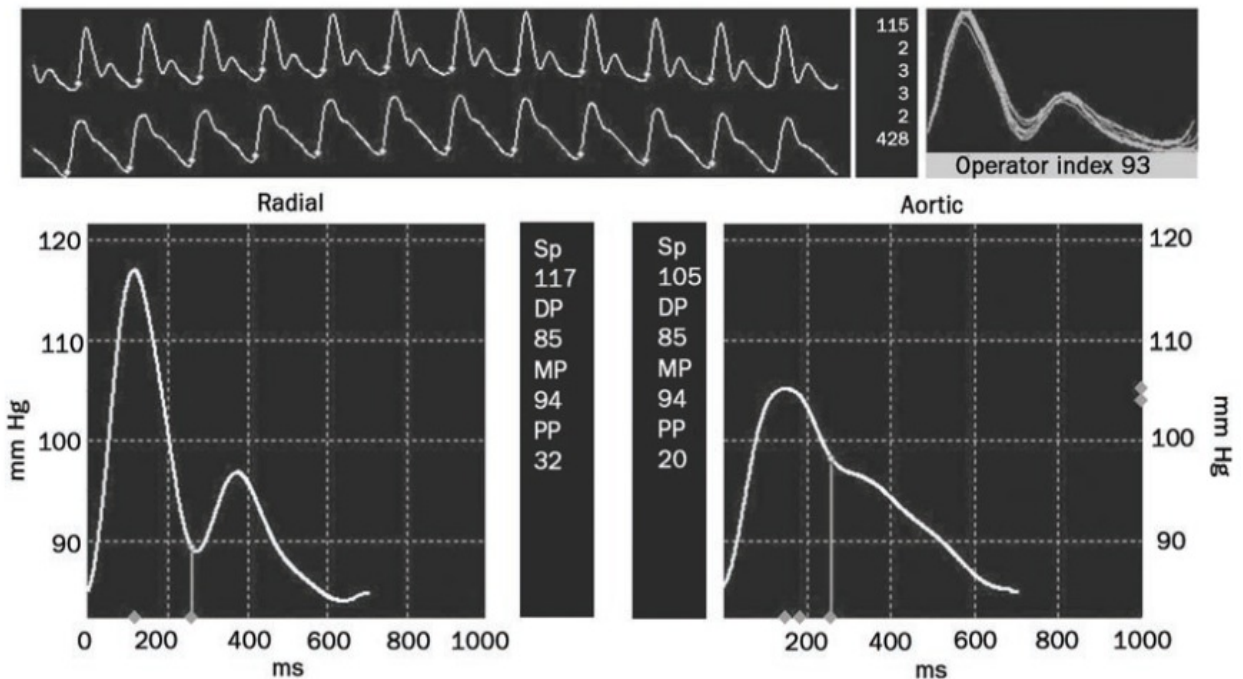
Patients were supine with the right arm extended, at heart level, and immobilized with foam supports at an angle of  $90^\circ$  from the torso. After an initial rest period of 10 minutes, applanation tonometry was performed by placing a handheld tonometer (strain gauge pressure sensor (AtCor Medical Pty Ltd, Sydney, Australia)) over the radial artery, applying pressure to flatten the artery (figure 3.1). Tonometry requires that the artery to be flattened against a hard surface (bone) [122]. Ten completely consistent radial waveforms are then recorded and ensembled into a single waveform (figure 3.2). The radial artery pressure is then recorded digitally and a generalized transfer function, approved by the food and drug administration [24], is used to calculate central pressure indices and produce the central waveform (figure 3.2). The SBP and DBP in the radial artery were calibrated after entering BP values obtained from the brachial pressure, using an automated cuff (Omron Healthcare Inc.) prior to data acquisition. Central SBP, DBP, and PP are all measured from the peak and trough data obtained from the waveform (Figure 3.3).





**Figure 3.1.** Measurement of Alx, PWV, central PP, and BPs with applanation tonometry.

This figure illustrates the use of a handheld tonometer by placing it over the radial artery. A radial waveform is produced and a generalized transfer function is used to calculate central pressure indices and produce the central waveform.



**Figure 3.2.** Radial artery applanation tonometry recording.

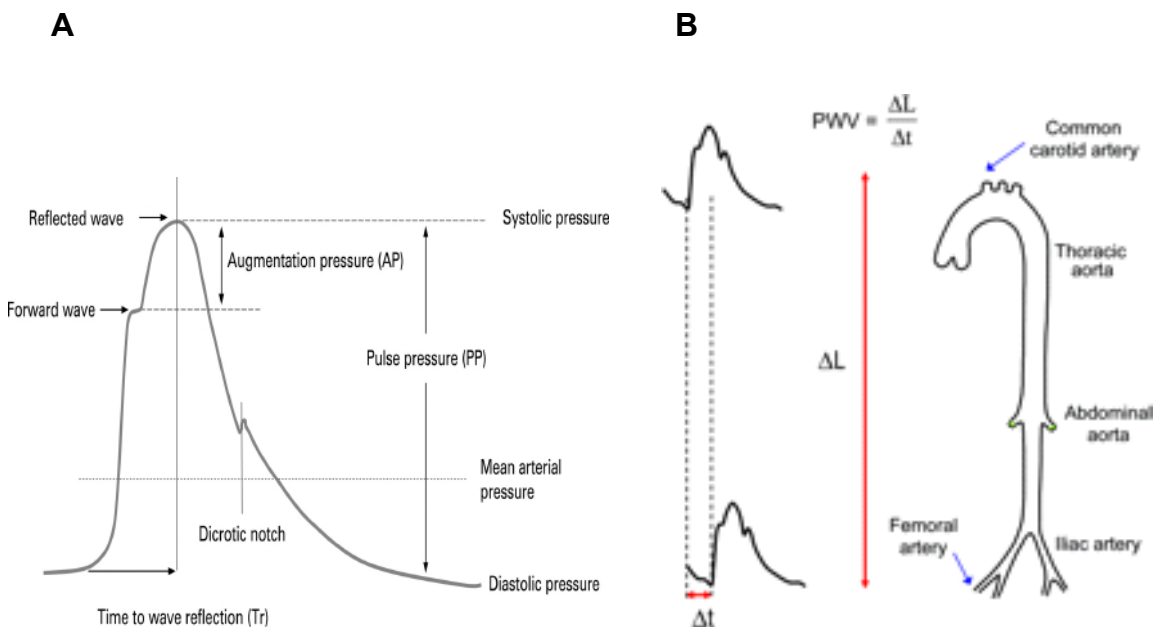
The upper long panel shows the radial pressure waveform above the derived central pressure waveform. The bottom left panel demonstrates a magnified radial arterial waveform. The bottom right panel provides a magnified derived central pressure waveform. Both lower panels show SBP, DP, MAP, and PP.

Alx was determined from applanation tonometry of the right radial artery with the SphygmoCor system (AtCor Medical Pty Ltd, Sydney, Australia) [122], using the central pressure waveform (figure 3.3.A). Alx is the ratio between AP (additional pressure added to the forward wave by the reflected wave) to the central PP and is expressed as a percentage [24]. For between-group comparison, Alx was normalized to a HR of 75 beats per minute (bpm). All measurements were subjected to quality control by the software and only high-quality recordings, defined as an operator index 80% were included. The MetS group had an average index of  $90 \pm 3$ , compared to  $95 \pm 2$  for the controls.

### **3.5.2. Pulse Wave Velocity**

PWVcf was measured by applanation tonometry, using the SphygmoCor AtCor Medical Pty. Ltd., Sydney, Australia [123]. The SBP and DBP in the peripheral and central arteries were calibrated after entering BP values obtained from the brachial pressure, using an automated cuff (Omron Healthcare Inc.), prior to data acquisition. ECG-gated carotid and femoral artery waveforms were sequentially recorded as described early in the radial artery. Aortic length (L) was calculated as the difference in the distances (in meters (m)) from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the femoral artery. The time delay ( $t$ , seconds) between the onset of carotid and femoral waveforms (foot-to-foot) was determined from the onset of the waveform and the R wave, recorded on the ECG during the cardiac cycles (figure 3.3.B). PWV was calculated as the ratio of distance to time ( $PWV = L/t$  (m/s)). The

PWV<sub>cr</sub> was measured using the same tonometry technique. The length was calculated as the difference in distances from the carotid sampling site to the suprasternal notch and from the suprasternal notch to the radial artery. To establish the day-to-day reproducibility of PWV measurements in our laboratory, we performed a study on 7 subjects of varying age for 3 consecutive testing days. The coefficients of variation were 9.0% and 4.3% for PWV<sub>cf</sub> and PWV<sub>cr</sub>, respectively.



**Figure 3.3.** Central waveform and ECG-gated carotid and femoral artery waveforms

(A) SBP & DP are the peak and trough of the waveform. AP is the additional pressure added to the forward wave by the reflected wave. Alx is the ratio between AP and cPP. The reflected wave in this central pressure waveform results in augmentation of systolic flow. (B) Aortic length (L) was calculated as the difference in the distances (in meters (m)) from the carotid site to suprasternal notch and from the suprasternal notch to the femoral artery. The time delay ( $t$ , seconds) between the onset of carotid and femoral waveforms (foot-to-foot) was determined from the onset of the waveform and the R wave, recorded on the ECG during the cardiac cycles (Figure 3.4). PWV was calculated as a ratio of distance to time ( $PWV = L/t$  (m/s)).

### **3.5.3 Intima-Media Thickness**

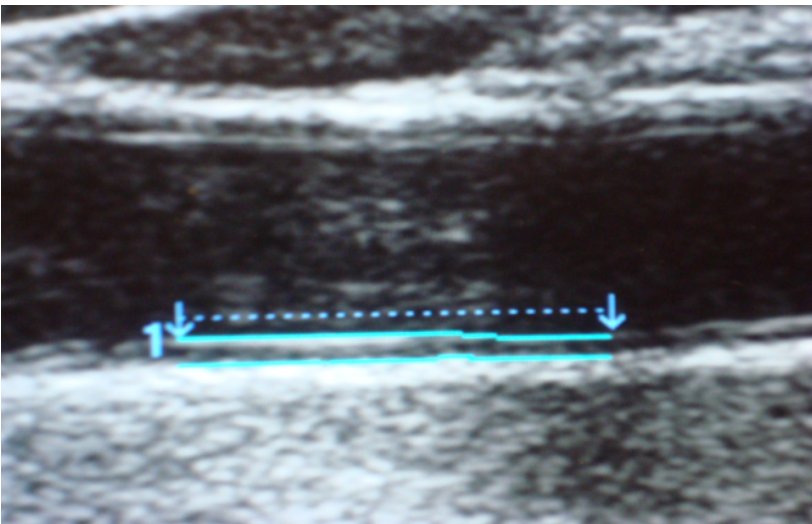
Carotid artery IMT was measured from the images derived from an ultrasound machine (GE vividi) equipped with a high-resolution linear array transducer. All subjects were in a supine position with a slight hyperextension and rotation of the neck in the direction opposite the probe. Longitudinal two-dimensional ultrasound images of the right common carotid artery were obtained by placing the probe in the anterior position of the neck, at approximately 1-2 cm proximal to the carotid bifurcation (figure 3.4). The images were recorded for approximately 45 seconds and stored for later off-line analysis.

IMT is defined as the distance between the leading edges of the lumen-intima and media-adventitia interface [124]. Images of the anterior IMT were measured, 1 cm proximal to the bifurcation (figure 3.5), during diastole, using the ECG as a reference point (end-diastolic phase is between the P and Q wave) (figure 3.5). Every third cardiac cycle was recorded for a total of 6 measurements and the average was computed to produce the IMT value. A single reader who was blinded to the clinical characteristics of the patients did all offline measurements.



**Figure 3.4.** Ultrasound probe recording carotid IMT.

Longitudinal two-dimensional ultrasound images of the right common carotid artery were obtained, by placing the probe in the anterior position of the neck, at approximately 1-2 cm proximal to the carotid bifurcation.



**Figure 3.5.** IMT measurement.

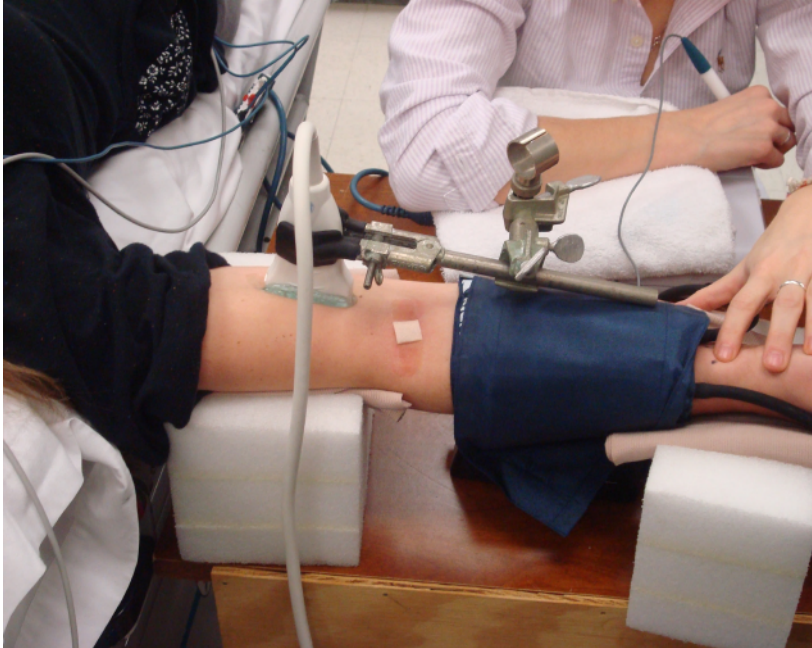
Images of the anterior IMT were measured, 1 cm proximal to the bifurcation during diastole. Every third cardiac cycle was recorded for a total of 6 measurements and the average was computed to produce the IMT value.

#### **3.5.4. Flow Mediated Dilation**

Patients rested supine with the right arm extended and immobilized with foam supports at an angle of 90° from the torso. HR was continuously monitored with a 3-lead ECG. A rapid inflation and deflation pneumatic cuff was positioned on the imaged arm immediately distal to the antecubital fossa to provide a stimulus to forearm ischemia (figure 3.6). A 10-MHz multifrequency linear array probe, attached to a high-resolution ultrasound machine (GE Vividi), was used to image

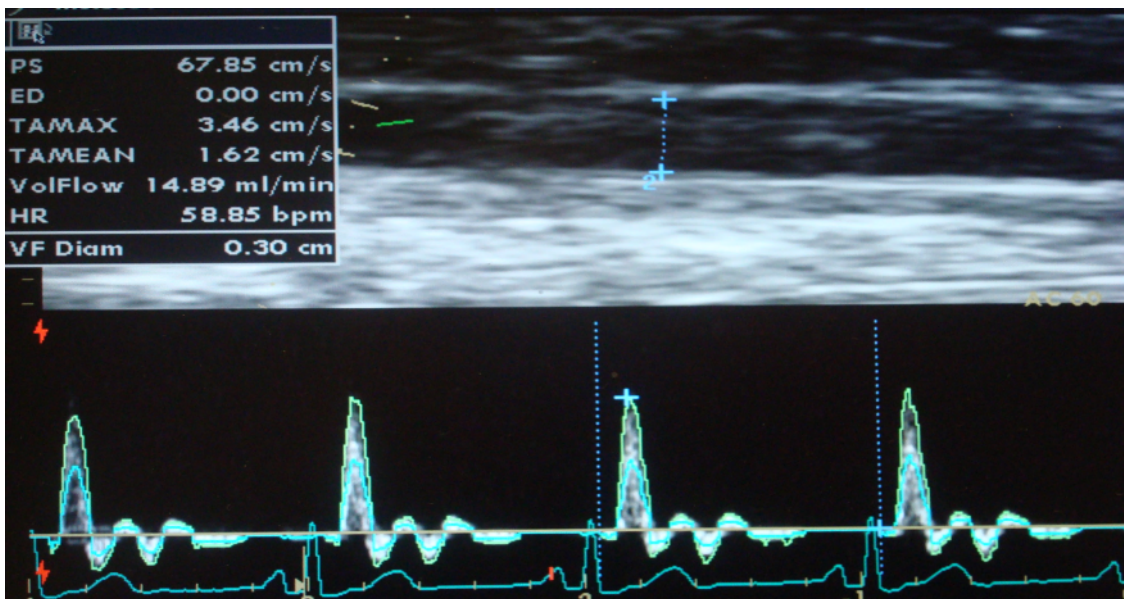
the brachial artery in the distal third of the upper arm. Duplex ultrasound was used for simultaneous acquisition of B-mode diameter and Doppler velocity signals, to use for offline assessment of change in diameter and flow velocity, respectively [125]. The probe was held stable in a stereotactic clamp (figure 3.6). After baseline images were obtained, the cuff was inflated to 250 mmHg for 5 minutes. Following limb ischemia there is a rapid increase in forearm blood flow, which slowly returns to baseline values, and is termed reactive hyperemia [125]. Digitized images of the right brachial artery (figure 3.7) were captured continuously for 30 seconds before cuff inflation and for 5 minutes following cuff release, to document the endothelial-dependent vasodilator response.

The arterial diameter was measured from the recordings captured during the FMD response (figure 3.7). Measurements were acquired from visual inspection by placing ultrasonic calipers from the anterior to the posterior wall. For the reactive hyperemia scan, diameter measurements were taken every 3<sup>rd</sup> cardiac cycle for the entire recorded duration; and the measurements were averaged. Arterial diameters in scans after reactive hyperemia were expressed as percentages of the first control scan [ $((\text{peak diameter} - \text{average resting diameter}) / \text{average resting diameter}) \times 100\%$ ]. Flow was calculated from Doppler velocity and artery diameter. Reactive hyperemia was calculated as the maximum flow recorded after cuff deflation divided by the flow during the resting baseline [ $((\text{peak velocity} - \text{average resting velocity}) / \text{average resting velocity}) \times 100\%$ ]. The mean artery diameter and percent dilatation were obtained by averaging the measurements taken over all occasions on which that patient was studied.



**Figure 3.6.** FMD technique used to assess endothelial-dependent vasodilation.

Patients rested with the right arm extended and immobilized with foam supports at an angle of 90° from the torso. A pneumatic cuff was positioned on the arm distal to the antecubital fossa to provide a stimulus to forearm ischemia.



**Figure 3.7.** Recordings and measurement of the right brachial artery during FMD.

Digitized images of the brachial artery, captured continuously for 30 seconds before cuff inflation and for 5 minutes beginning 30 seconds before cuff deflation to document the endothelial-dependent vasodilator response. The arterial diameter was measured from the recordings captured during the FMD response. Measurements were taken from the anterior to the posterior wall every 3<sup>rd</sup> cardiac cycle for the entire recorded duration and averaged.

Percent change in FMD was normalized to shear rate area under the curve (FMD:SR<sub>AUC</sub>) to control for the presence of the large inter-subject variability in reactive hyperemia-induced shear stress. Shear rate (an estimate of shear stress) was calculated as 4 x mean blood velocity/arterial diameter [125]. Pike et al [126] concluded that the SR<sub>AUC</sub>, not peak shear, as the critical determinant of the FMD response, and should be used for normalization. Peak shear rate represents only the portion of FMD response immediately following cuff release, where as SR<sub>AUC</sub> represents the shear stimuli responsible for the entire duration of post-cuff release, up till the peak diameter change; calculated for each individual.

### **3.6. Metabolic Risk Score**

The relationships between the Metabolic Risk Score (MRS) and select baseline aspects of metabolic and hemodynamic parameters, and arterial function were also investigated. It was not originally an aim of our study to look at the effect of the MRS on arterial stiffness or remodeling. However, after observing significant improvement in several measurements, and other changes that just failed to reach significance, we felt it was important to observe participants who were very close to being classified as either having the MetS or a healthy control, on a continuous risk score. Eight additional participants, who presented with only 2 out of the 5 criteria for MetS were included in the MRS regression analysis (n=44).

The diagnostic score was created by a group, Macchia et al [127], who exploited the individual diagnostic components of the MetS aimed at predicting the risk of late-onset diabetes to be easily used in clinical practice. They assigned points to



each risk factor (table 3.1), which were weighted proportionally to the value of the B-coefficients of multivariate analysis (including metabolic parameters that were categorized as statistical quintiles, relative to time-to-diabetic event). The MRS is then obtained by summing all the individual points.

Table 3.1. Metabolic risk factors and risk point valuation.

| <b>Criteria</b>  | <b>Risk Points</b> |
|--|--------------------|
| Men  | 3                  |
| Age >50 years  | 4                  |
| Hypertension (systolic >135 mmHg,<br>Diastolic >85 mmHg) | 2                  |
| BMI (kg x m <sup>-2</sup> )                              |                    |
| <26  | 0                  |
| 26-27  | 3                  |
| ≥28  | 6                  |
| Triglycerides (mg/dl)                                    |                    |
| <100   | 0                  |
| 100-159  | 5                  |
| 160-199  | 7                  |
| ≥200   | 9                  |
| HDL Cholesterol (mg/dl)                                  |                    |
| ≥50  | 0                  |
| 30-49  | 3                  |
| <30  | 5                  |
| Fasting Glucose (mg/dl)                                  |                    |
| <80  | 0                  |
| 80-99  | 5                  |
| 90-99  | 10                 |
| 100-109  | 16                 |
| ≥110   | 28                 |

### **3.7. Statistics**

All data are presented as Means  $\pm$  SEM. Data from each experiment were first tested for normality (Kolmogorov-Smirnov test). Normally distributed data were analyzed for statistically significant differences using parametric tests. Simple comparisons between two independent cases were analyzed using Student's two-tailed independent t-test. Comparisons between the same data sets were analyzed using Paired Sample t-test. More complex comparisons were conducted using repeated measures analysis of variance followed by Turkey's HSD post-hoc analysis.

Linear regression analyses were performed to determine the association among variables. In all cases, MRS was used as the predictor variable. Pearson product-moment correlation coefficients were used to indicate the magnitude and direction of relations among variables. The slopes of the regression lines (comparing MRSs and the dependent variable) were compared using analysis of covariance, which included an interaction term.

In general, all analyses were performed with SPSS Statistics 20 (SPSS Inc, Chicago, IL, U.S.A.). P-values  $<0.05$  were considered statistically significant.

# **Chapter 4**

## **Results**

## 4.1. Baseline Characteristics of Study Population

Comparisons of baseline characteristics between MetS individuals (n=21) and healthy controls (n=15) are displayed in table 4.1. The MetS participants were not significantly different in age than controls. Our data also showed that there is no significant difference in height ( $171 \pm 2$  vs.  $169 \pm 2$  cm) or HR, where the MetS group averaged  $69 \pm 2$  bpm compared to  $73 \pm 4$  bpm for the controls.

MetS individuals weighed significantly ( $p < 0.001$ ) more ( $236 \pm 9$  vs.  $153 \pm 8$  pounds;  $p < 0.001$ ), had a higher BMI ( $36.8 \pm 1.3$  vs.  $24.3 \pm 1.1$  kg x m<sup>-2</sup>;  $p < 0.001$ ) and body fat % ( $35.1 \pm 1.3$  vs.  $23.3 \pm 1.7$  %,  $p < 0.001$ ) relative to the healthy controls.

All of the criteria for the MetS defined by the NCEP ATP III [55], were significantly different in the two groups, except for HDL cholesterol. Although not significant HDL cholesterol was lower in the MetS individuals ( $43.4 \pm 3.5$  mg/dl) compared to healthy controls ( $52.0 \pm 3.6$  mg/dl). This shows that the study population was a true representation of the MetS. Within the group of individuals with the MetS, 62% had hypertension, 86% had abdominal obesity, 43% had elevated triglyceride levels, 71% had low HDL cholesterol concentration, and 53% had high fasting plasma glucose.

Table 4.1. Clinical characteristics of 21 MetS individuals and 15 healthy controls

| <b>Variables</b>                        | <b>Metabolic Syndrome (n=21)</b> | <b>Controls (n=15)</b> | <b>P Value</b> |
|---|----------------------------------|------------------------|----------------|
| Age (years)                             | 47 ± 3                           | 41 ± 3                 | 0.14           |
| Men (%)                                 | 43%                              | 40%                    | -              |
| Height (cm)                             | 171 ± 2                          | 169 ± 2                | 0.468          |
| Weight (pounds)                         | 236 ± 9                          | 153 ± 7                | <0.001         |
| Body Mass Index (kg x m <sup>-2</sup> ) | 36.8 ± 1.3                       | 24.3 ± 1.1             | <0.001         |
| Body Fat (%)                            | 35.1 ± 1.3                       | 23.3 ± 1.7             | <0.001         |
| Waist (cm)                              | 116 ± 3                          | 83 ± 3                 | <0.001         |
| Triglycerides (mg/dl)                   | 163 ± 15                         | 92 ± 12                | 0.001          |
| HDL Cholesterol (mg/dl)                 | 43.4 ± 3.5                       | 52.0 ± 3.6             | 0.104          |
| Serum Glucose (mg/dl)                   | 100 ± 2                          | 95 ± 2                 | 0.040          |
| Heart Rate (beats/min.)                 | 67 ± 2                           | 73 ± 4                 | 0.311          |
| Brachial SBP (mm Hg)                    | 133 ± 3                          | 115 ± 3                | <0.001         |
| Brachial DBP (mm Hg)                    | 83 ± 2.0                         | 73 ± 2                 | 0.001          |
| Brachial MAP (mm Hg)                    | 102 ± 2                          | 89 ± 2                 | <0.001         |
| Brachial PP (mm Hg)                     | 49 ± 3                           | 47 ± 4                 | 0.639          |
| Central SBP (mm Hg)                     | 123 ± 3                          | 104 ± 2                | <0.001         |
| Central DBP (mm Hg)                     | 85 ± 2                           | 83 ± 5                 | 0.600          |
| Central PP (mm Hg)                      | 38 ± 3                           | 29 ± 1                 | 0.018          |

Data are expressed as mean ± SEM for n = 36. Significant difference between MetS individuals and controls at p<0.05.

The MetS group had significantly ( $p < 0.001$ ) higher PWVcf ( $8.0 \pm 0.3$  m/s) than the control group ( $6.4 \pm 0.3$  m/s) (figure 4.1A). The PWVcr (figure 4.1B) was not significantly ( $p = 0.61$ ) different between the MetS individuals ( $8.0 \pm 0.3$  m/s) and control group ( $7.9 \pm 0.2$  m/s). After adjusting for MAP, PWV remained significantly ( $p < 0.001$ ) elevated in the MetS compared to controls.

An interesting finding was the baseline characteristic detailing the concept of pressure amplification and differences of stiffness in different arterial segments. The MetS group had a significantly ( $p < 0.05$ ) higher cPP (figure 4.2A) of  $38.2 \pm 3.5$  mmHg compared to the control group ( $28.8 \pm 1.4$  mmHg). However, the brachial PP (bPP) (figure 4.2B) was not significantly different between the MetS individuals ( $48.9 \pm 3.0$  mmHg) and control group ( $46.5 \pm 4.1$  mmHg).

In addition to PWV and cPP measures of arterial stiffness, The MetS group had significantly ( $p < 0.05$ ) higher carotid IMTmax ( $0.72 \pm 0.04$  mm) than the control group ( $0.61 \pm 0.02$  mm) (figure 4.3A). The carotid IMTavg (figure 4.3B) was also higher in the MetS group ( $0.60 \pm 0.03$  vs.  $0.53 \pm 0.02$  mm), although it fell slightly short of reaching significance ( $p = 0.057$ ).

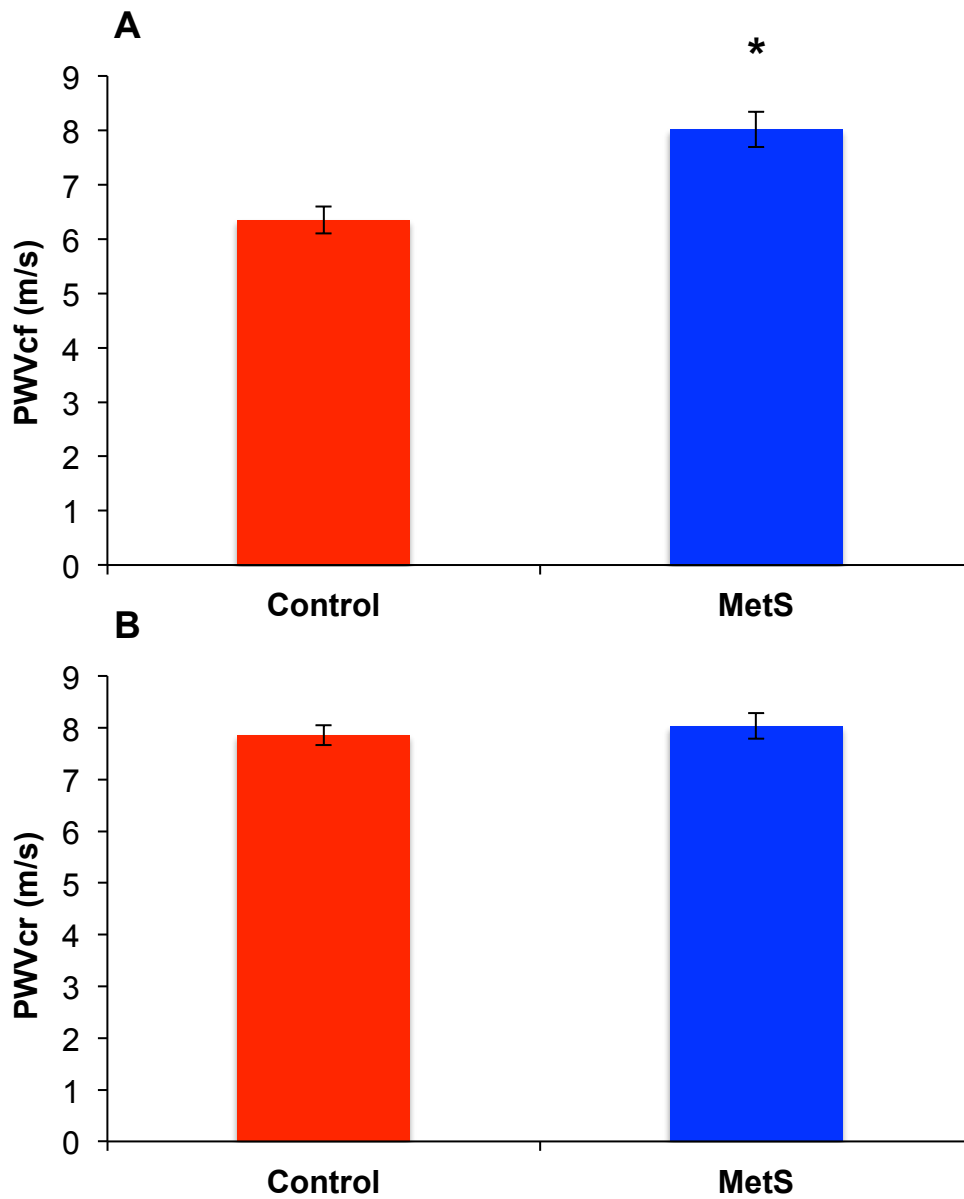


Figure 4.1. Mean comparison of carotid-femoral (A) and carotid-radial (B) PWV between controls and MetS. Data are expressed as mean  $\pm$  SEM. Significant difference between the two at \* $p < 0.001$ .

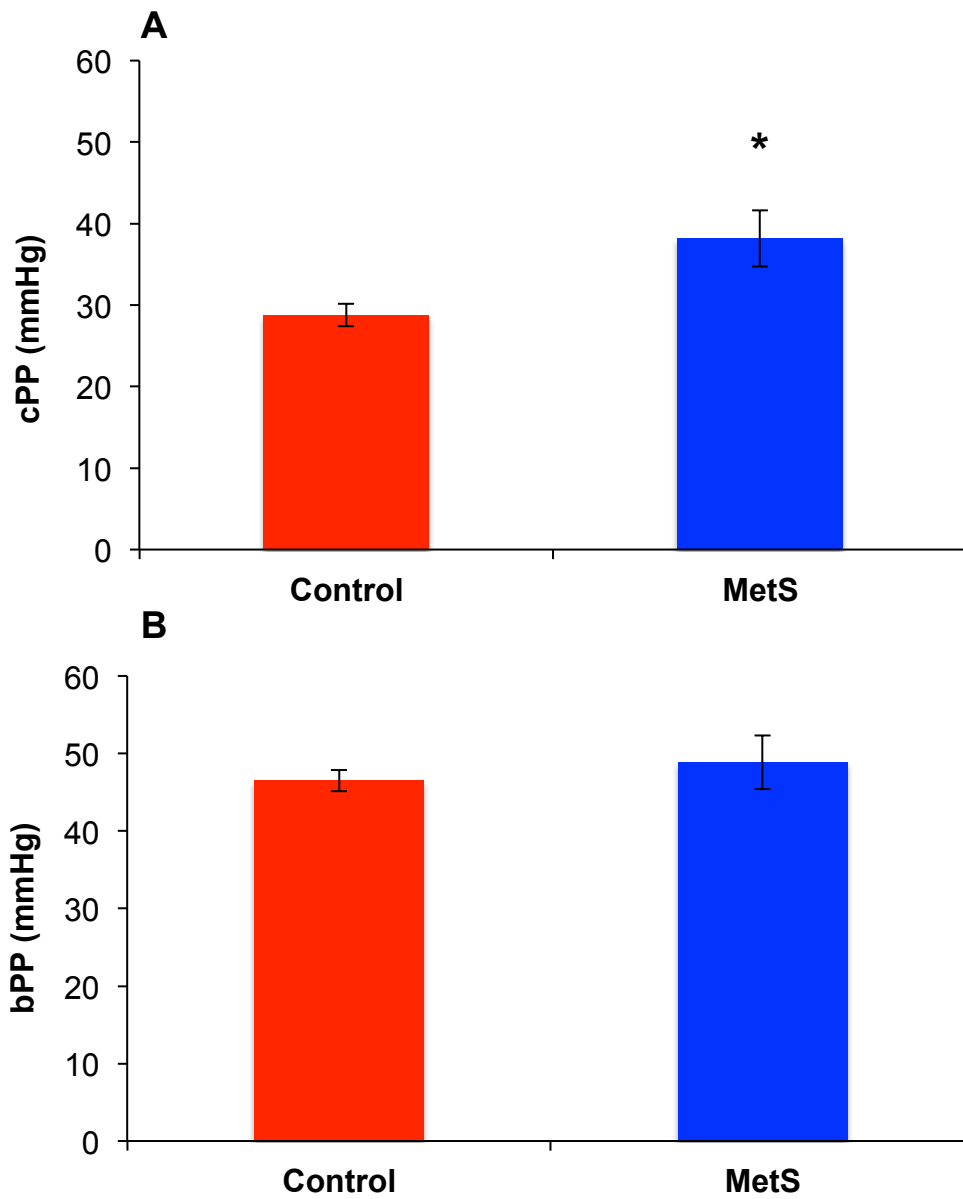


Figure 4.2. Mean comparison of cPP (A) and bPP (B) between controls and MetS. Data are expressed as mean  $\pm$  SEM. Significant difference between the two at  $*p < 0.05$ .



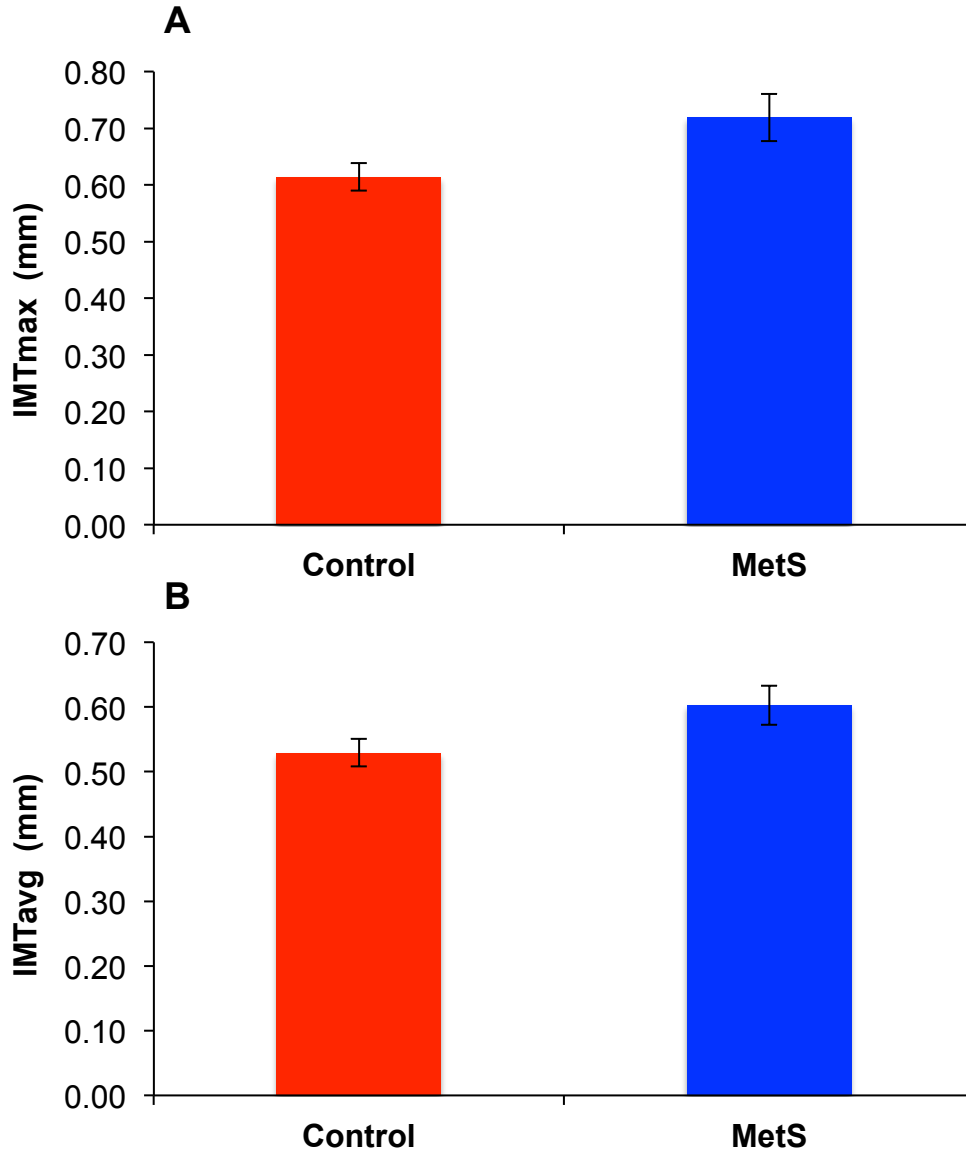


Figure 4.3. Mean comparison of maximum (A) and average (B) IMT between controls and MetS. Data are expressed as mean  $\pm$  SEM. Significant difference between the two at \* $p < 0.05$ .

Our data showed that the MetS group had a differential impact on wave reflection (figure 4.4A) of  $23.8 \pm 2.9\%$  compared to  $18.3 \pm 4.2\%$  for the controls, although significance was not established. When corrected for HR, the  $\text{Alx}@75$  (figure 4.4B) was not different in the MetS group ( $18.4 \pm 2.7\%$ ) compared to the control group ( $12.8 \pm 4.1\%$ ), while significance again was not reached.

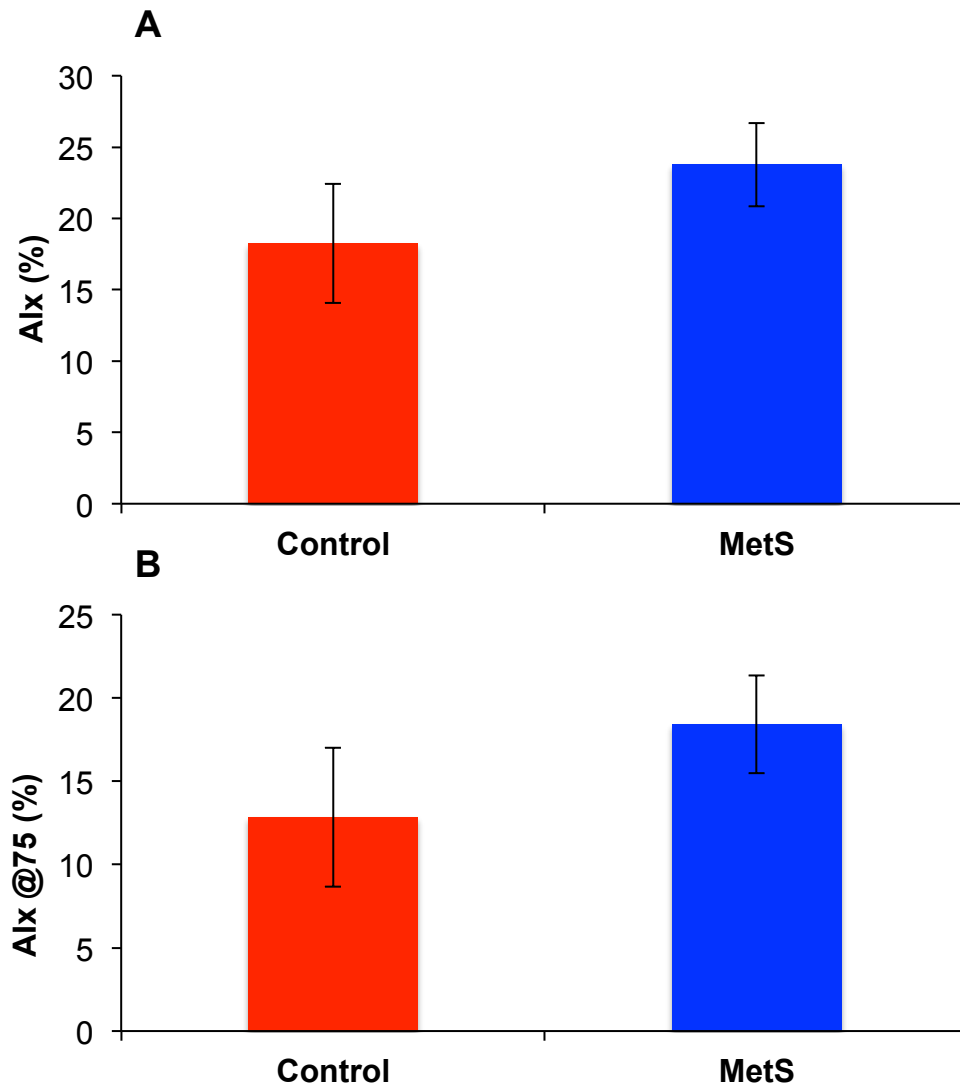


Figure 4.4. Mean comparison of Alx (A) and Alx@75 (B) between controls and MetS. Data are expressed as mean  $\pm$  SEM.  $p=0.27$  and  $p=0.25$ , respectively.

In addition to arterial function changes, peak aerobic capacity, measured as  $VO_2$ peak (figure 4.5) was also significantly ( $p<0.01$ ) lower in the MetS group ( $17.1 \pm 1.1$  ml/kg/min) than the control group ( $23.3 \pm 1.6$  ml/kg/min).

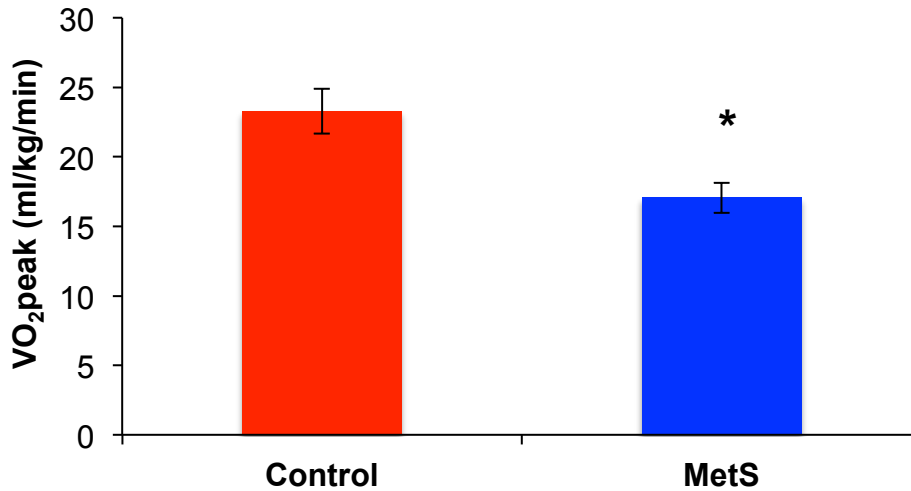


Figure 4.5. Mean comparison of  $VO_2$ peak between controls and MetS. Data are expressed as mean  $\pm$  SEM. Significant difference between the two at  $p<0.01$ .

Our data showed no significant difference in measures of endothelial function between the MetS group and healthy controls. The MetS group had  $7.4 \pm 0.9$  % change in FMDdia (figure 4.6A) compared to healthy controls that had a  $7.8 \pm 1.5$  % change. The MetS individuals had a FMDflow % change (figure 4.6B) of  $1452 \pm 167\%$  and the controls a  $1887 \pm 263$  % change. Finally, when normalized for  $SR_{AUC}$  (figure 4.6C) the FMDdia % change between the MetS and healthy control group remained insignificant ( $1.8 \times 10^{-4} \pm 2.5 \times 10^{-5}$  vs.  $2.3 \times 10^{-4} \pm 4.1 \times 10^{-5}$  % change).

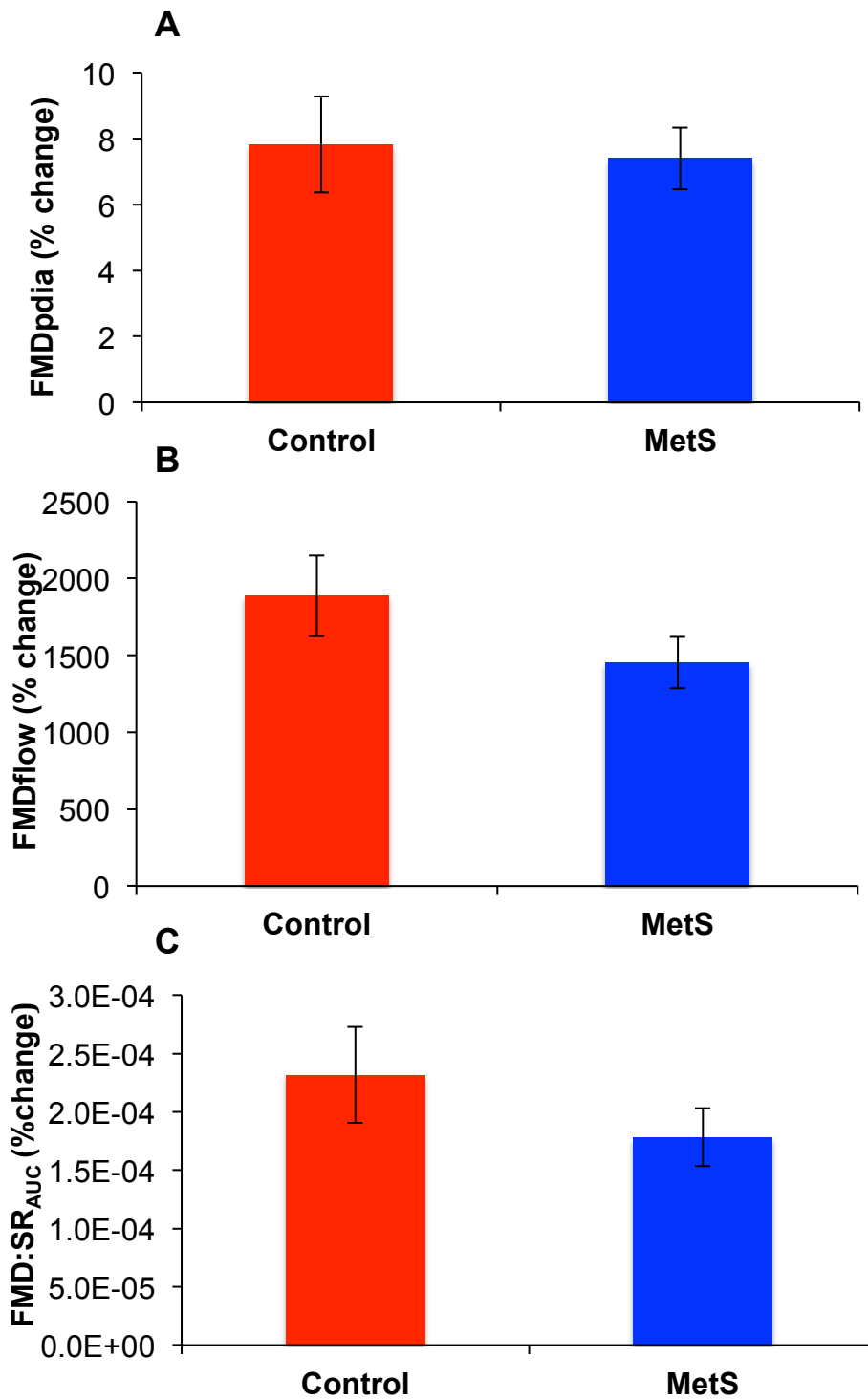


Figure 4.6. Mean comparison of FMDdia % change (A), FMDflow % change (B), and FMD:SR<sub>AUC</sub> (C) between controls and MetS. Data are expressed as mean ± SEM. p=0.80, p=0.15, and p=0.25, respectively.

## 4.2. Effects of Aerobic Exercise on Measures of Arterial Stiffness

Of the 21 MetS individuals, 11 underwent 8 weeks of aerobic exercise training for a period of one-hour, 3 times a week. Eight other MetS individuals maintained their normal daily lifestyle and performed no physical activity during this period of time. The two remaining MetS individuals included in the baseline analysis did not complete the exercise protocol and follow-up testing.

There were no significant differences at baseline (table 4.2) between the MetS individuals who performed exercise and the sedentary group of MetS participants in every aspect of metabolic, hemodynamic, and anthropometric measurement; except for HDL cholesterol which was higher in the trained group ( $50.9 \pm 5.5$  mg/dl) compared to non-trained participants ( $47.4 \pm 4.3$ ), reaching significance ( $p < 0.05$ ).

There was no significant baseline difference of carotid-femoral or carotid-radial PWV between the MetS trained and non-trained group. The exercise-trained group (figure 4.7A) had a significant ( $p < 0.05$ ) decrease in PWV<sub>cf</sub> ( $8.1 \pm 0.5$  vs.  $7.2 \pm 0.4$  m/s), while the non-trained group experienced no significant change ( $8.0 \pm 0.5$  vs.  $7.8 \pm 0.3$  m/s). The PWV<sub>cr</sub> (figure 4.7B) did not change significantly for either the trained group ( $7.9 \pm 0.4$  vs.  $8.0 \pm 0.3$  m/s) or the non-trained individuals ( $8.1 \pm 0.1$  vs.  $7.7 \pm 0.3$  m/s).

Table 4.2. Baseline clinical characteristics of 11 MetS trained individuals and 8 MetS non-trained Individuals before and after exercise training or non-training.

| Variables                               | MetS Trained (n=11) |            | MetS Non-Trained (n=8) |            | Compared before exercise | Compared after exercise |
|---|---------------------|------------|------------------------|------------|--------------------------|-------------------------|
|   | Before              | After      | Before                 | After      |                          |                         |
| Age (years)                             | 46 ± 4              | -          | 47 ± 3                 | -          | 0.723                    | 0.723                   |
| Men (%)                                 | 27%                 | -          | 50%                    | -          | 0.993                    | 0.982                   |
| Height (cm)                             | 168 ± 3             | -          | 174 ± 2                | -          | 0.138                    | 0.138                   |
| Weight (pounds)                         | 239 ± 9             | 238 ± 11   | 239 ± 8                | 238 ± 13   | 0.589                    | 0.415                   |
| Body Mass Index (kg x m <sup>-2</sup> ) | 38.7 ± 1.8          | 38.6 ± 1.8 | 35.8 ± 1.5             | 35.6 ± 1.6 | 0.274                    | 0.765                   |
| Body Fat (%)                            | 36.6 ± 1.4          | 36.4 ± 1.4 | 33.4 ± 2.6             | 34.1 ± 2.9 | 0.286                    | 0.837                   |
| Waist (cm)                              | 118 ± 4             | 118 ± 4    | 113 ± 3                | 113 ± 3    | 0.194                    | 0.121                   |
| Triglycerides (mg/dl)                   | 137 ± 18            | 175 ± 24   | 175 ± 24               | 161 ± 28   | 0.620                    | 0.111                   |
| HDL Cholesterol (mg/dl)                 | 50.9 ± 5.5          | 47.4 ± 4.3 | 47.4 ± 4.3             | 33.0 ± 4.9 | <0.05                    | 0.992                   |
| Serum Glucose (mg/dl)                   | 99 ± 2              | 97 ± 2     | 97 ± 3                 | 85 ± 12    | 0.307                    | 0.281                   |
| Heart Rate (beats/min.)                 | 65 ± 3              | 63 ± 3     | 63 ± 3                 | 67 ± 4     | 0.292                    | 0.669                   |
| Brachial SBP (mm Hg)                    | 129 ± 5             | 126 ± 4    | 126 ± 4                | 124 ± 2    | 0.776                    | 0.120                   |
| Brachial DBP (mm Hg)                    | 80 ± 3              | 82 ± 3     | 82 ± 3                 | 81 ± 2     | 0.440                    | 0.109                   |
| Brachial MAP (mm Hg)                    | 98 ± 2              | 97 ± 3     | 97 ± 3                 | 101 ± 6    | 0.354                    | 0.991                   |
| Brachial PP (mm Hg)                     | 49 ± 3              | 44 ± 2     | 47 ± 4                 | 43 ± 2     | 0.970                    | 0.612                   |
| Central SBP (mm Hg)                     | 119 ± 4             | 116 ± 4    | 116 ± 4                | 112 ± 3    | 0.877                    | 0.135                   |
| Central DBP (mm Hg)                     | 82 ± 2              | 81 ± 3     | 81 ± 3                 | 82 ± 2     | 0.451                    | 0.321                   |
| Central PP (mm Hg)                      | 37 ± 6              | 34 ± 3     | 34 ± 3                 | 31 ± 2     | 0.582                    | 0.936                   |

Data are expressed as mean ± SEM. P values represent differences in means at baseline, significance between trained and non-trained Individuals at p<0.05.

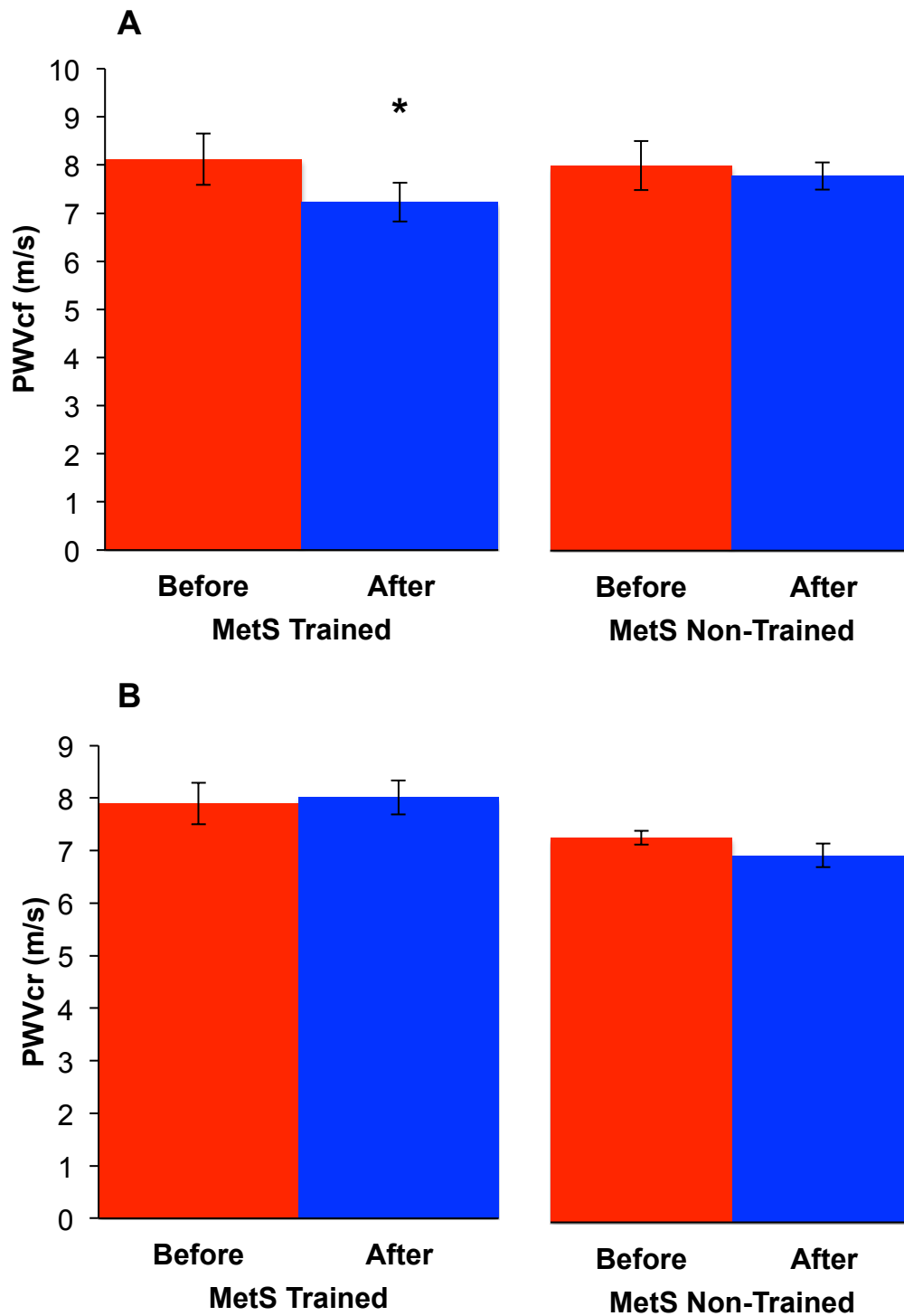


Figure 4.7. Mean comparison of carotid-femoral (A) and carotid-radial (B) PWV in MetS trained vs. non-trained individuals before and after 8 weeks of aerobic exercise training. Data are expressed as mean  $\pm$  SEM. Significant difference before and after the 8-week intervention, \* $p < 0.05$ , within each group.

There was no significant baseline difference between IMTavg or IMTmax between the MetS trained and non-trained group. The exercise-trained group (figure 4.8A) witnessed no significant change in IMTavg ( $0.58 \pm 0.03$  vs.  $0.58 \pm 0.03$  mm), and the non-trained group also had no significant change ( $0.59 \pm 0.05$  vs.  $0.61 \pm 0.05$  mm). IMTmax (figure 4.8B) did not change significantly for the trained group ( $0.69 \pm 0.04$  vs.  $0.69 \pm 0.03$  mm) or the non-trained individuals ( $0.71 \pm 0.08$  vs.  $0.71 \pm 0.05$  mm).

There was no significant baseline difference in cPP between the MetS trained and non-trained group. Following 8-weeks of aerobic exercise training cPP (figure 4.9A) in the trained group decreased ( $37.4 \pm 6.0$  vs.  $33.5 \pm 2.7$  mmHg), although not significantly. The non-trained group also had a decrease in cPP ( $34.3 \pm 1.7$  vs.  $30.8 \pm 1.7$  mmHg), which was also found not to be significant.

Alx was analyzed with PWA to observe the effect of aerobic exercise train on wave reflections. After normalizing Alx to heart rate of 75, there was no significant difference in Alx@75 (figure 4.9B) at baseline between the Mets trained group ( $18.2 \pm 3.5$  %) and non-trained individuals ( $14.0 \pm 5.0$  %). Following the 8 weeks of exercise the trained group decreased their Alx@75 to  $14.6 \pm 4.7$  %, which just fell short of reaching significance ( $p=0.075$ ). The non-trained group had no significant change in Alx@75 ( $14.0 \pm 5.0$  vs.  $13.5 \pm 4.3$  %).



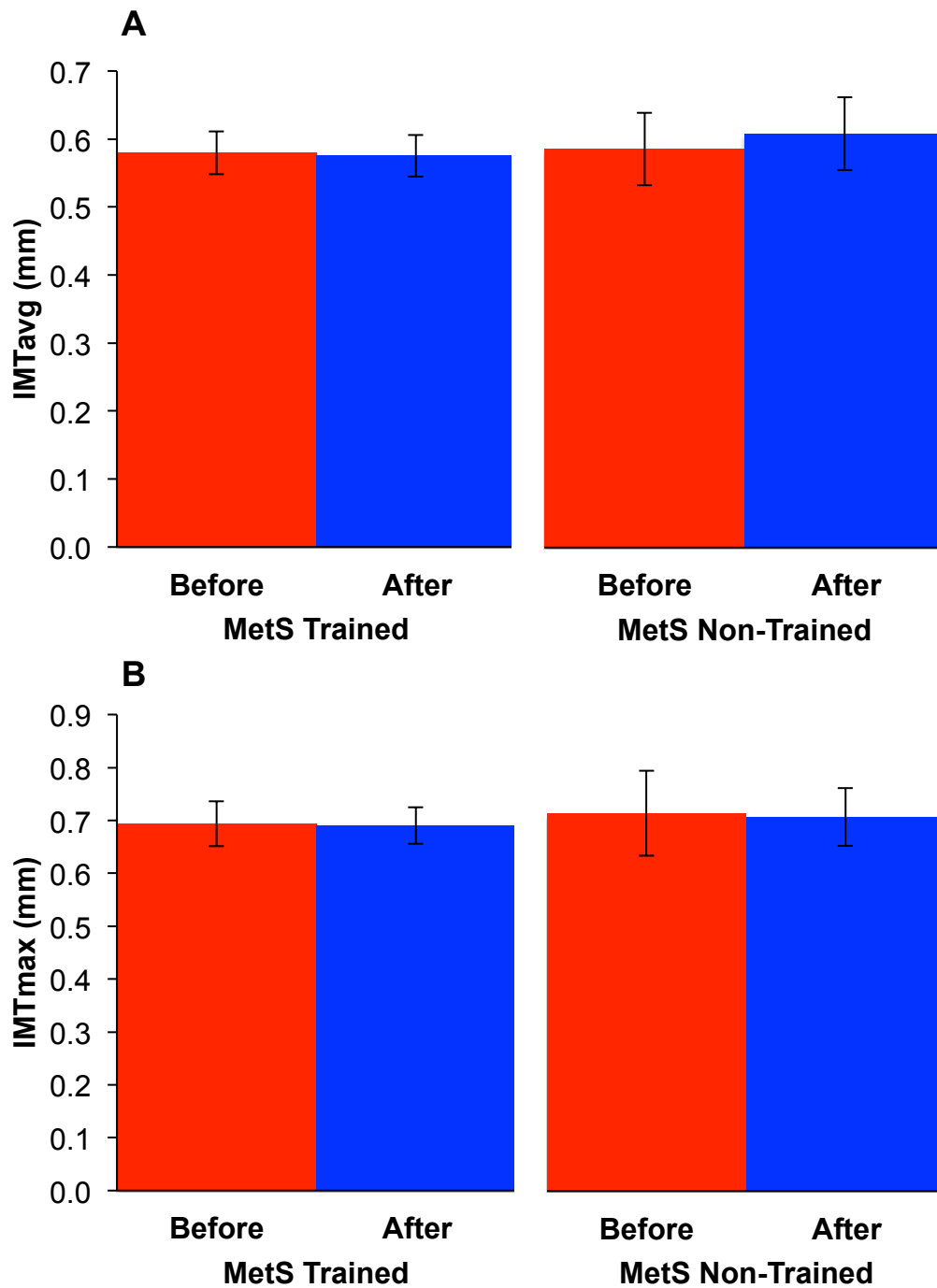


Figure 4.8. Mean comparison of average (A) and maximum (B) carotid IMT in MetS trained vs. non-trained individuals before and after 8 weeks of aerobic exercise training. Data are expressed as mean  $\pm$  SEM.

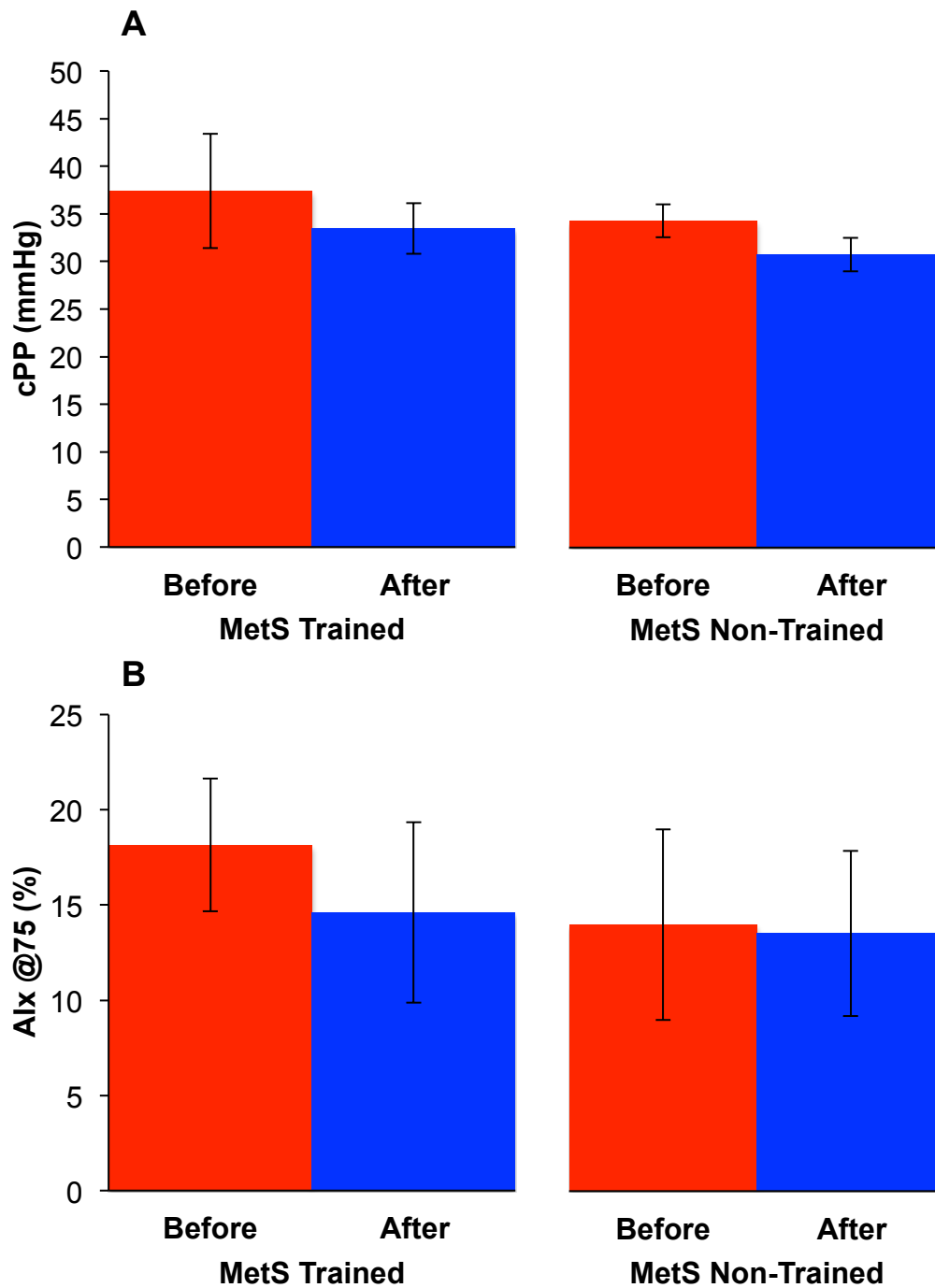


Figure 4.9. Mean comparison of central PP (A) and Alx@75 (B) in MetS trained vs. non-trained individuals before and after 8 weeks of aerobic exercise training. Data are expressed as mean  $\pm$  SEM.

Endothelial function was analyzed to observe the functional contribution of effects of aerobic exercise on arterial stiffness. The % change in FMDflow (figure 4.10A) was not significantly different between the trained ( $1579 \pm 282$  % change) and non-trained group ( $1298 \pm 200$  % change). Following 8 weeks of exercise, the trained group had a slight decrease in FMDflow ( $1437 \pm 258$  % change), but not significant. The non-trained group saw no significant change in % FMDflow ( $1298 \pm 200$  vs.  $1303 \pm 175$  % change). The % change in FMDpdia (figure 4.10B) was not significantly different at baseline in the non-trained MetS group ( $9.2 \pm 1.7$  %) compared to the trained group ( $6.6 \pm 1.0$  %). Following the 8 weeks of aerobic training, the trained group increased FMDpdia % change from  $6.6 \pm 1.0$  to  $8.1 \pm .59$  %, but not significantly. The non-trained group also had an increase, not significant, in FMDpdia % change ( $9.2 \pm 1.7$  vs.  $12.0 \pm 2.7$  % change). When normalized to shear rate (figure 4.10C) there was no significant difference at baseline between the trained and non-trained groups ( $1.6 \times 10^{-4} \pm 4.0 \times 10^{-5}$  vs.  $2.0 \times 10^{-4} \pm 1.9 \times 10^{-5}$ ). The non-trained group had no change after the 8 weeks ( $1.9 \times 10^{-4} \pm 7.1 \times 10^{-5}$ ), while the trained group had an increase ( $1.6 \times 10^{-4} \pm 4.0 \times 10^{-5}$  vs.  $2.2 \times 10^{-4} \pm 3.3 \times 10^{-5}$ ), although not significant.

There was no significant difference in  $VO_2$ peak (figure 4.11) at baseline, but was slightly higher in the non-trained group ( $19.0 \pm 1.9$  ml/kg/min) before the exercise intervention, compared to the trained group ( $16.0 \pm 1.4$  ml/kg/min). Following 8-weeks of exercising the trained group had a significant ( $p=0.05$ ) increase in peak aerobic capacity from  $16.0 \pm 1.9$  to  $18.2 \pm 1.7$  ml/kg/min. The non-trained group had no change in  $VO_2$ peak ( $19.0 \pm 1.9$  vs.  $18.7 \pm 1.3$  ml/kg/min).

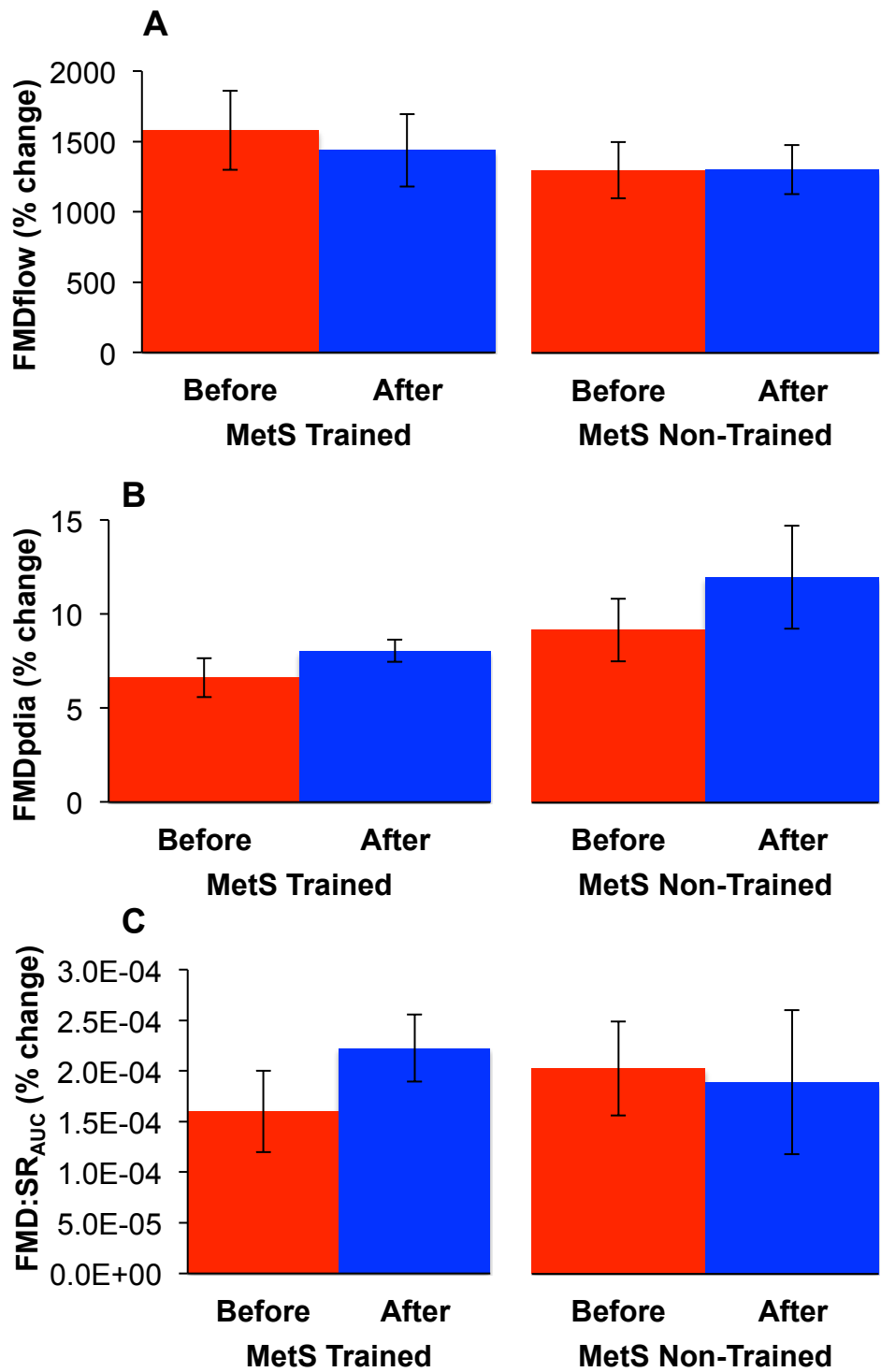


Figure 4.10. Mean comparison of FMDflow % change (A), FMDpdia % change (B), and FMD:SR<sub>AUC</sub> (C) in MetS trained vs. non-trained individuals before and after 8 weeks of aerobic exercise training. Data are expressed as mean ± SEM.

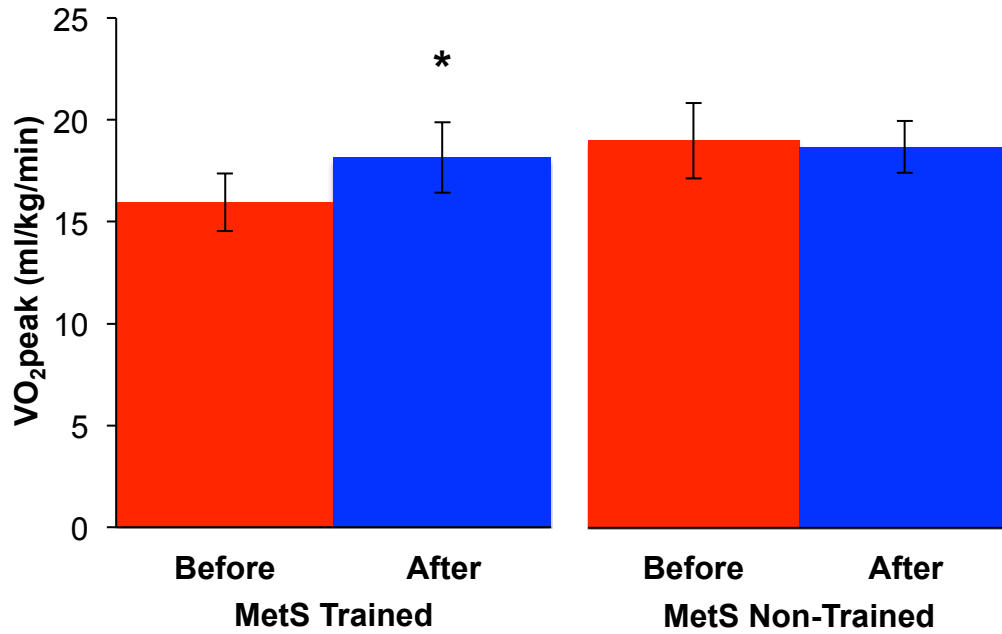


Figure 4.11. Mean comparison of VO<sub>2</sub>peak in MetS trained vs. non-trained individuals before and after 8 weeks of aerobic exercise training. Data are expressed as mean  $\pm$  SEM. Significant difference before and after the 8-week intervention, \*p=0.05, within each group.

### 4.3. Arterial Stiffness and Metabolic Risk Score

The relationships between the MRS and select baseline aspects of arterial dysfunction were also investigated. Eight additional participants, who presented with only 2 out of the 5 criteria for the MetS were included in the MRS regression analysis for an n=44. MRS and age were not correlated; therefore no statistical correction was performed.

Table 4.3 outlines the direct comparisons of the MRS to the MetS inclusion criteria. All indices were found to be significantly correlated with the MRS and evenly distributed, except for a lower negative correlation between the risk score and HDL cholesterol.

Table 4.3 Direct comparisons of the correlation coefficients for the MRS to all the MetS inclusion criteria.

|             | Waist  | Triglycerides | HDL Cholesterol | Glucose | SBP    |
|-------------|--------|---------------|-----------------|---------|--------|
| MRS R value | 0.61** | 0.62**        | -0.37*          | 0.66**  | 0.68** |

Significant Pearson's correlations at \*p<0.05, \*\*p<0.001

Initially, relationships of arterial stiffness assessments, with all participants combined, were investigated. The results revealed a significantly ( $p < 0.01$ ) positive correlation ( $r = 0.42$ ) of PWVcf to the MRS (figure 4.12A). Assessment of PWVcr (figure 4.12B) revealed no significant correlation with the MRS.

After assessment of arterial thickness; both average and maximum carotid IMT (figure 4.13) were found to be positively correlated ( $r = 0.38$  and  $r = 0.35$ , respectively) to the MRS; both measures reached significance ( $p < 0.05$ ).

Finally, cPP and Alx, adjusted for a heart rate of 75, were also assessed against the MRS. cPP and the MRS (figure 4.14A) were found to be positively correlated ( $r = 0.47$ ,  $p < 0.001$ ), but Alx@75 had no significant correlation to the MRS (figure 4.14B).

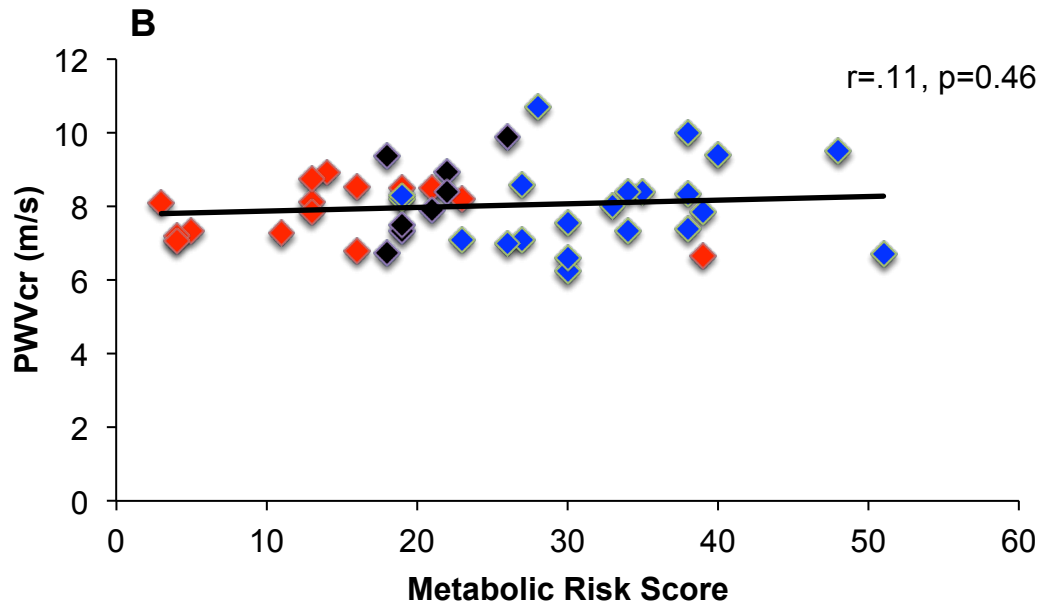
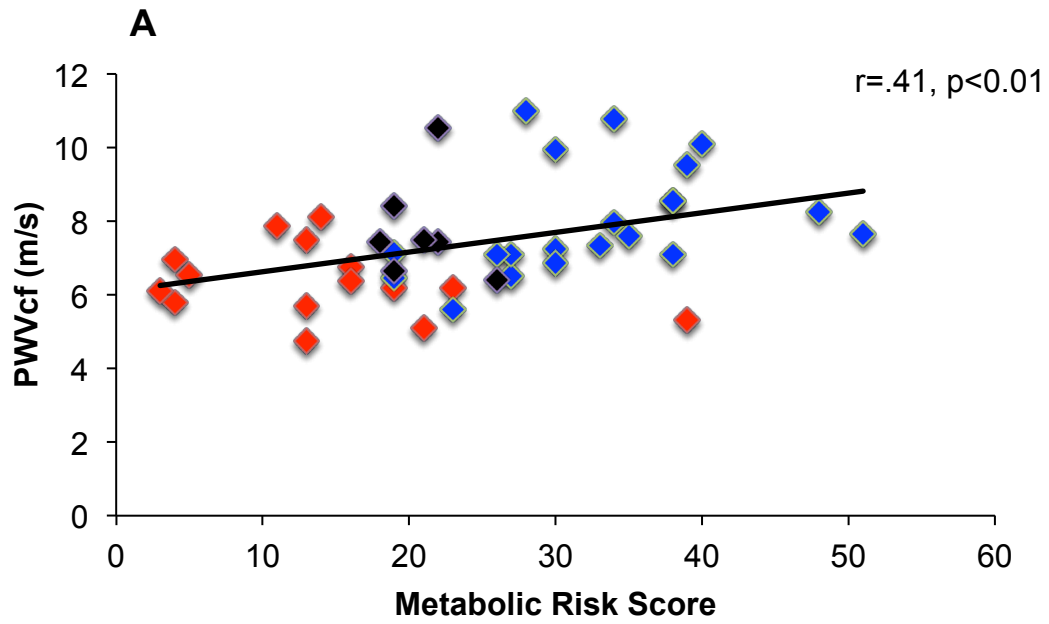


Figure 4.12. The relationships of PWVcf to MRS (A) and PWVcr to MRS (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).



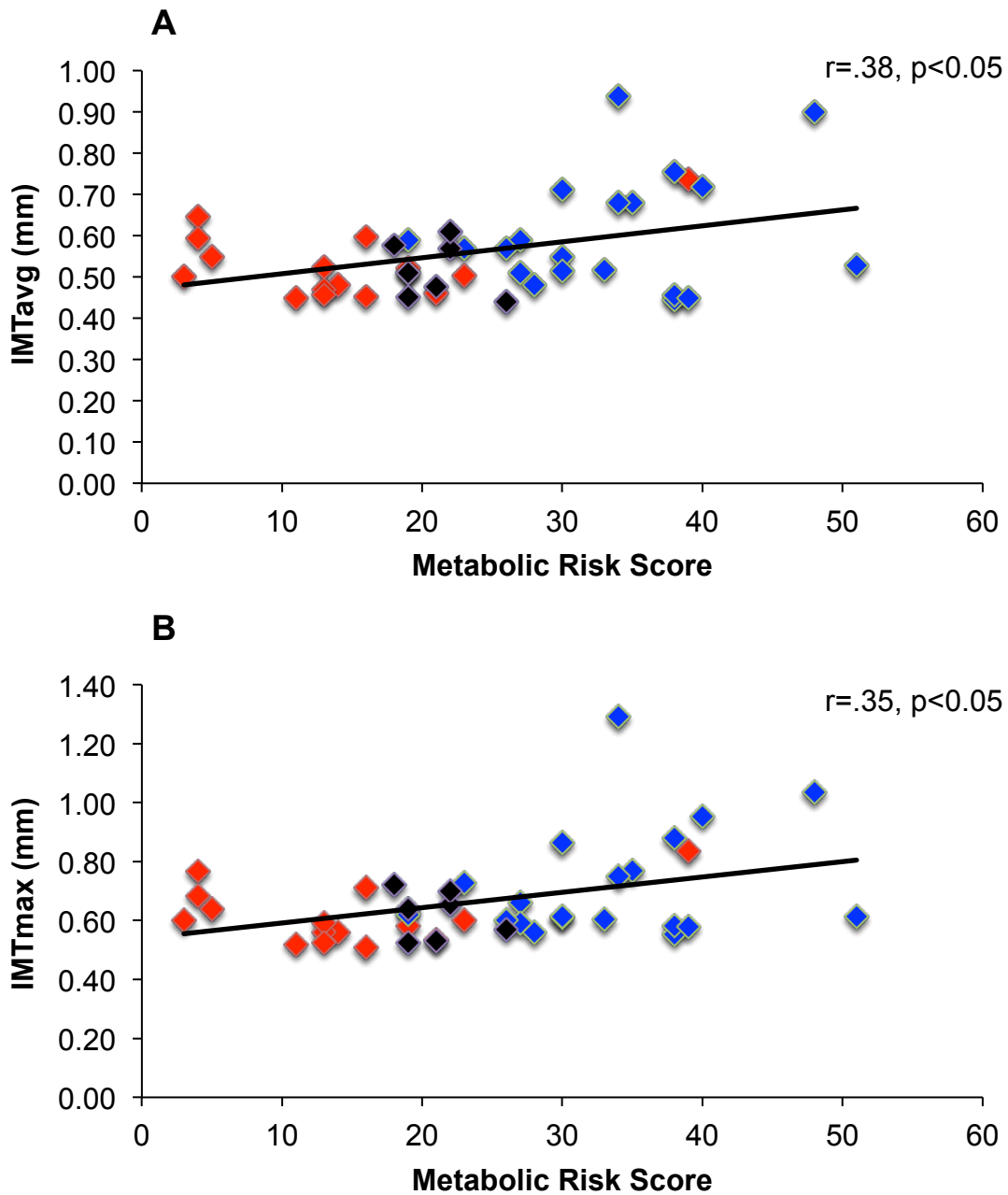


Figure 4.13. The relationships of IMTavg to MRS (A) and IMTmax to MRS (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

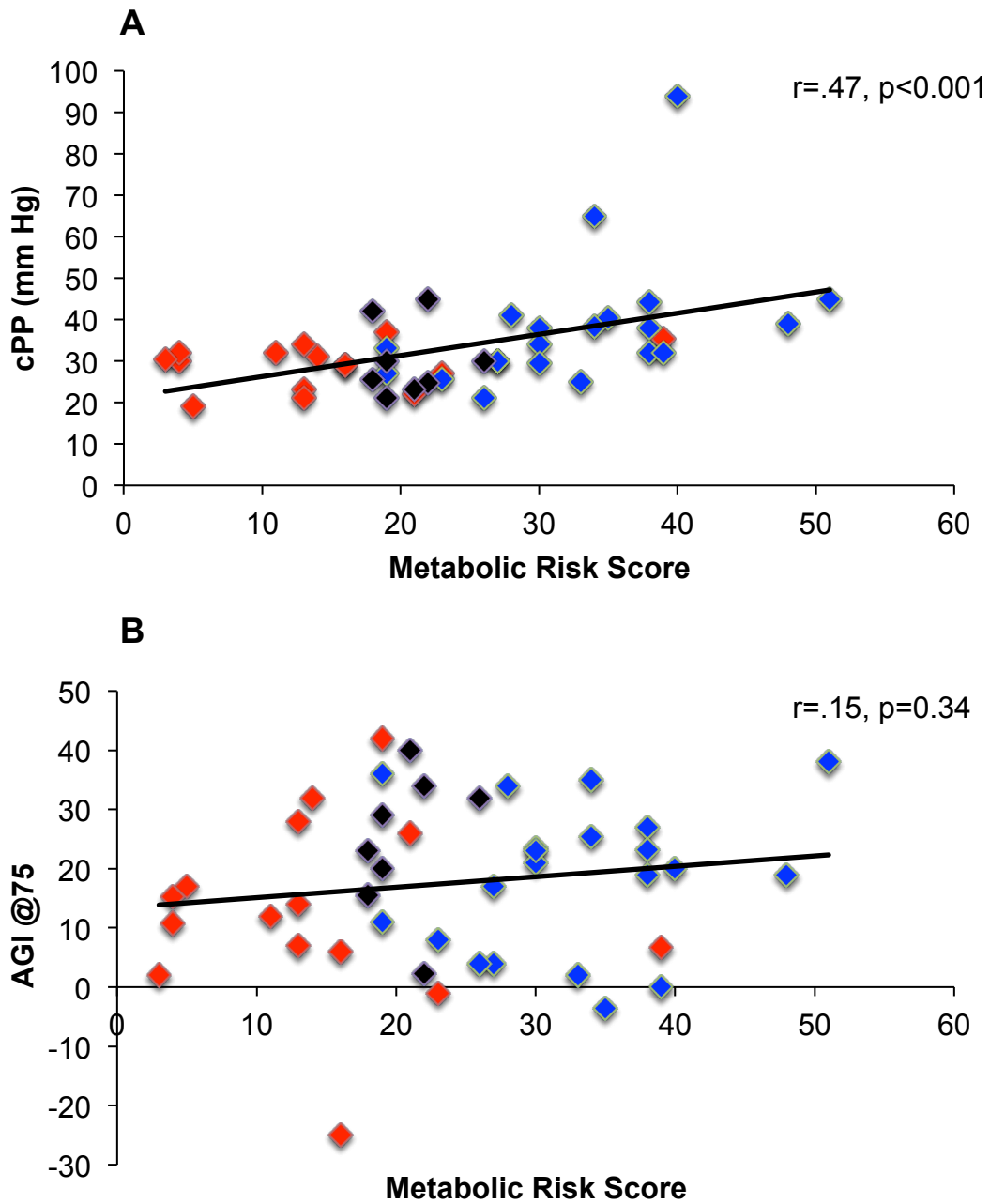


Figure 4.14. The relationships of cPP to MRS (A) and Alx@75 to MRS (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

After investigating the relationships between MRS and measures of arterial stiffness, we sought to confirm the relationships between measures of arterial stiffness and wall remodeling. First, the results revealed a significantly ( $p < 0.05$ ) positive correlation ( $r = .35$ ) between PWVcf and IMTavg (figure 4.15A). Assessment of PWVcf to IMTmax (figure 4.15B) also revealed a positive correlation ( $r = .38$ ,  $p < 0.05$ ). There was a strong correlation ( $r = .51$ ,  $p < 0.001$ ) of cPP to IMTavg (figure 4.16A). A positive correlation ( $r = .45$ ,  $p < 0.01$ ) was also found between cPP and IMTmax (figure 4.16B).

We looked at the relationship of PWVcf to indices of peripheral and central PPs to further study the degeneration of pulse pressure amplification with increased stiffening (figure 4.17). PWVcf was strongly correlated ( $r = .48$ ,  $p = 0.001$ ) to cPP, but was found to not be correlated with bPP.

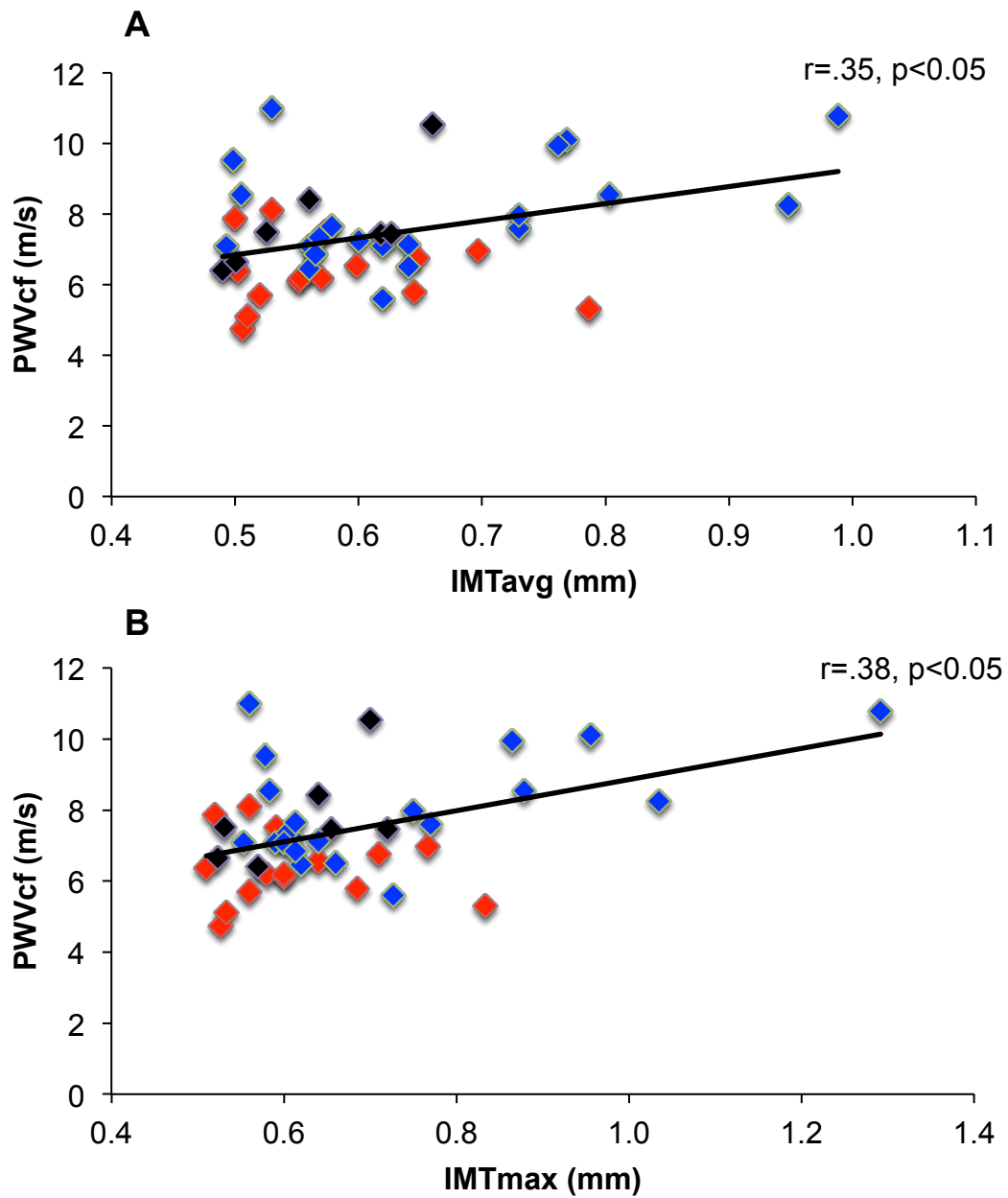


Figure 4.15. The relationships of PWVcf to IMTavg (A), and PWVcf to IMTmax (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

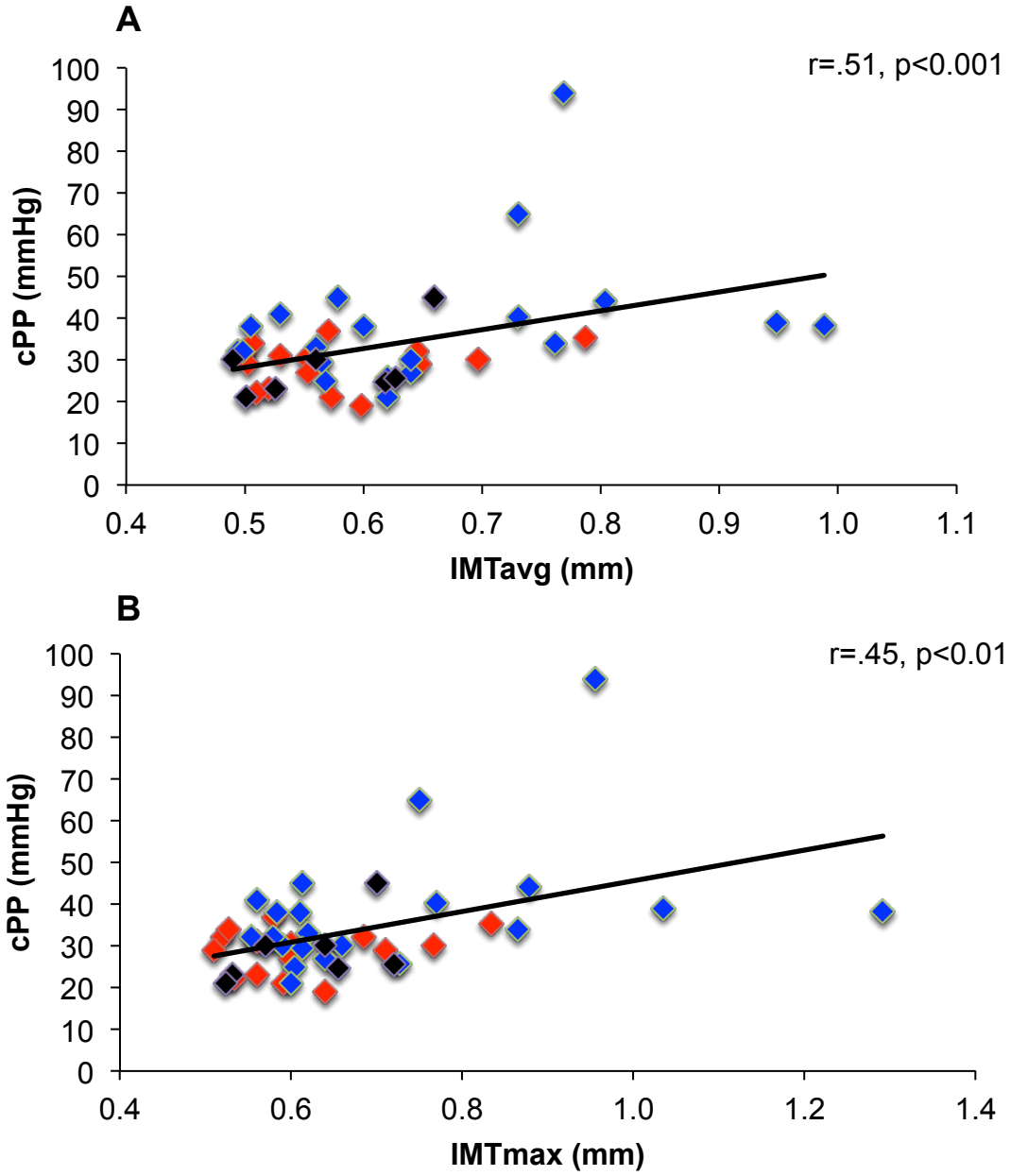


Figure 4.16. The relationships cPP to IMTavg (A), and cPP to IMTmax (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

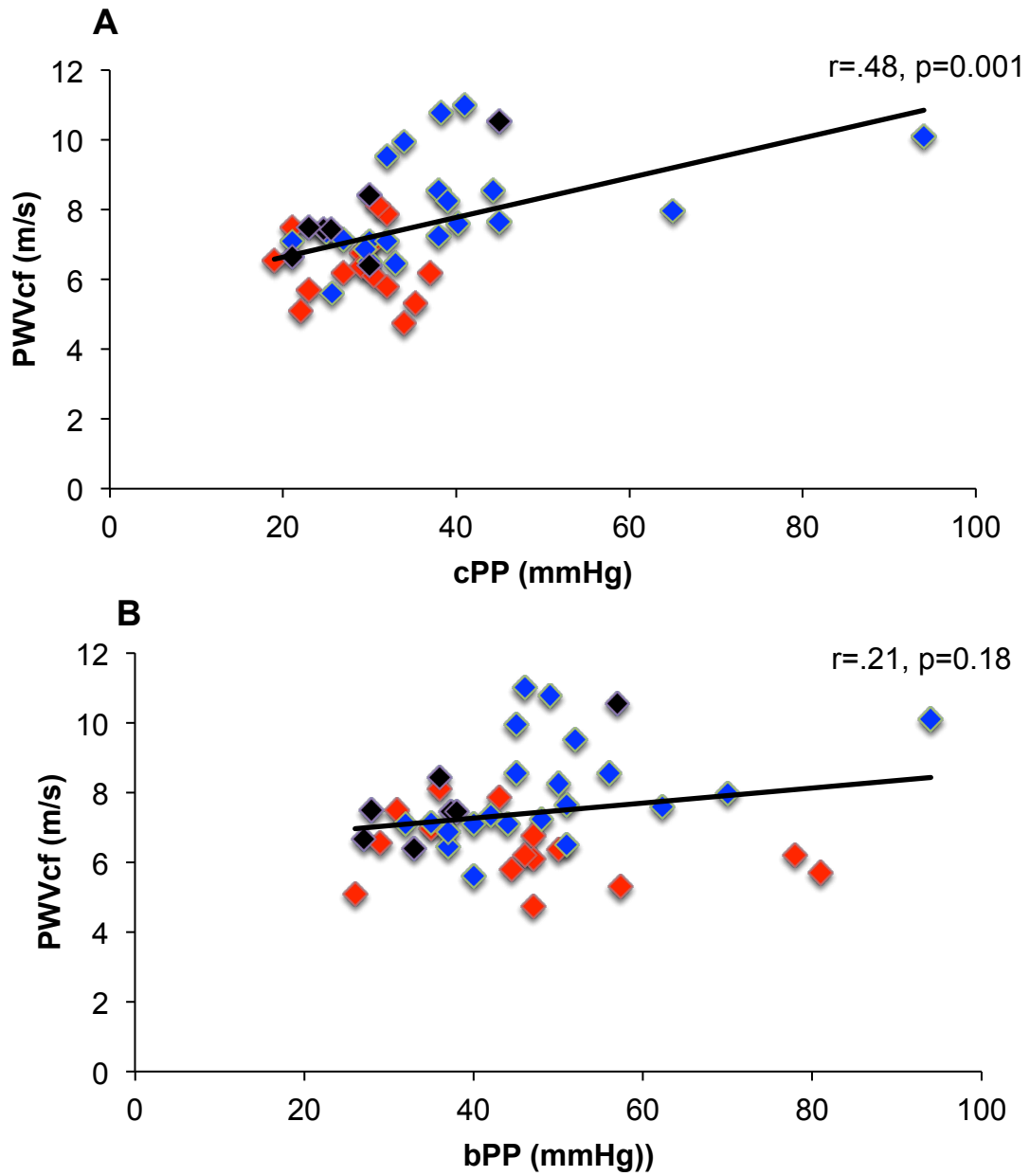


Figure 4.17. The relationships of PWVcf to cPP (A), and PWVcf to bPP (B) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

Our group was also interested in the relation of peak aerobic capacity to the MRS. We first discovered a strong negative correlation ( $r=-.40$ ,  $p<0.001$ ) of  $VO_{2peak}$  to the MRS (figure 4.18).

We then looked at the relationship between assessments of arterial stiffness and  $VO_{2peak}$ . A negative correlation ( $r=-.40$ ,  $p<0.01$ ) was found between PWVcf and  $VO_{2peak}$  (figure 4.19A). A negative correlation ( $r=-.43$ ,  $p<0.01$ ) was discovered with cPP to  $VO_{2peak}$  (figure 4.19B). Finally, IMTavg and  $VO_{2peak}$  (figure 4.19C) were also negatively correlated ( $r=-.41$ ,  $p<0.01$ ).

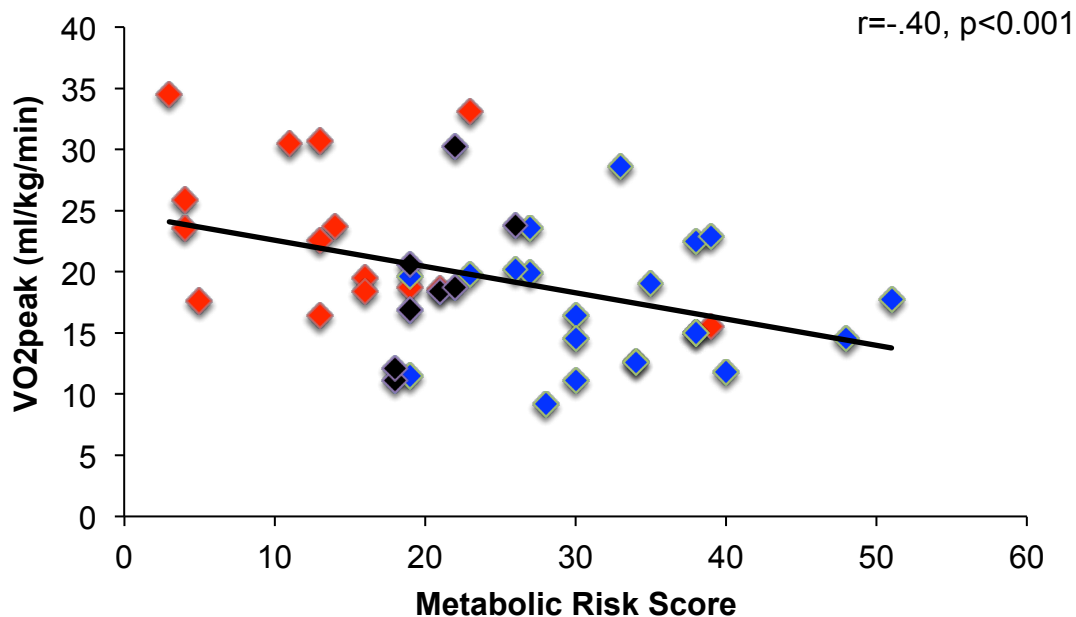


Figure 4.18. The relationship of  $VO_{2peak}$  correlated to MRS in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).

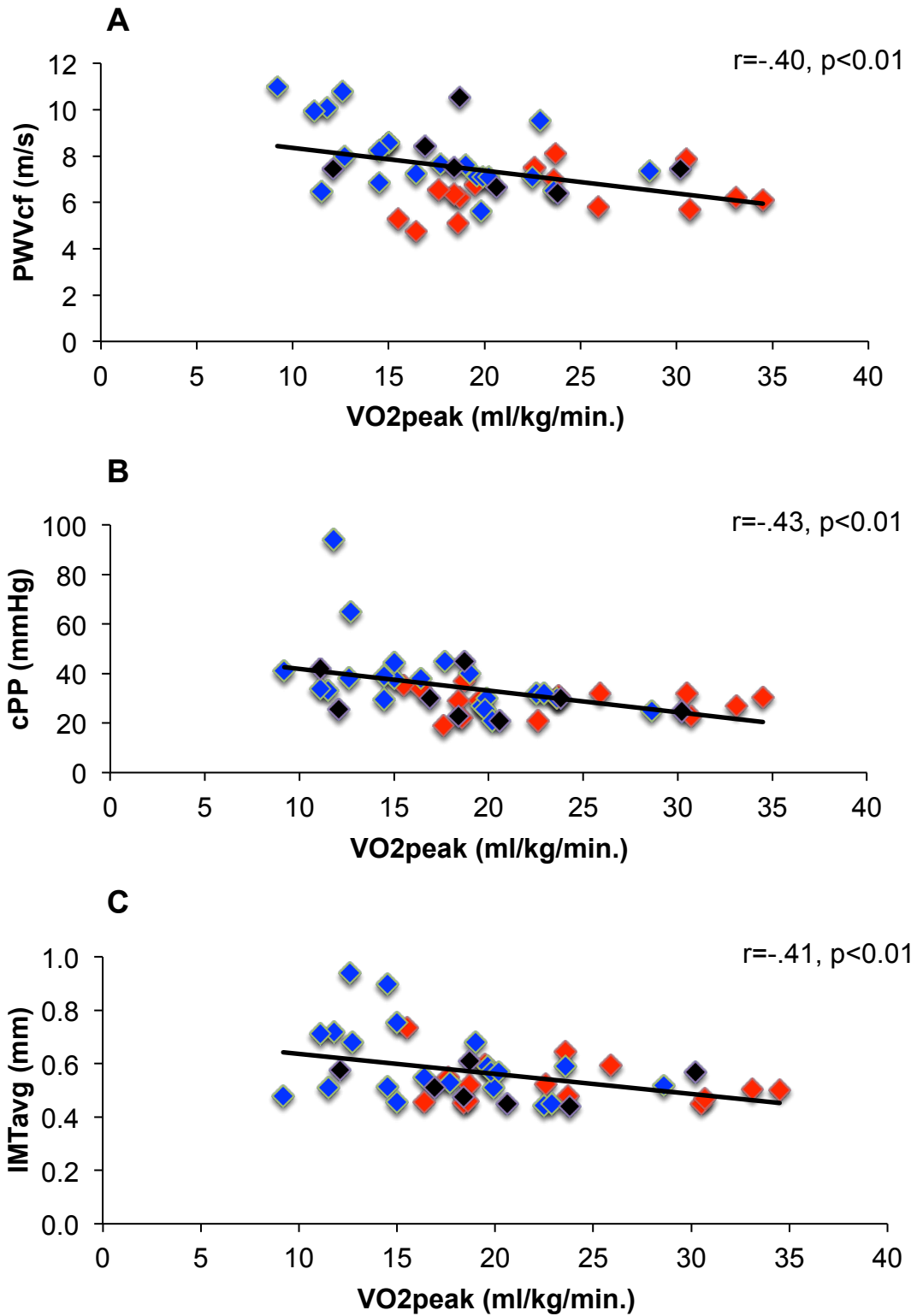


Figure 4.19. The relationships of PWVcf to VO<sub>2</sub>peak (A), cPP to VO<sub>2</sub>peak (B), and IMTavg to VO<sub>2</sub>peak (C) in controls (♦), MetS (♦), and those with 2 of the 5 MetS components (♦).



# **Chapter 5**

## **Discussion**

CVD is prevalent in industrialized societies, and metabolic disease is a dominant risk factor for morbidity and mortality [7]. Consequently, a primary public health concern is to understand the mechanisms by which the MetS is associated with CVD, and to establish appropriate prevention and intervention strategies. One putative mechanism that has emerged as an important risk factor for CVD is the increase in arterial stiffness in the MetS population [128]. This single degenerative alteration to the vasculature has profound effects on CV health, contributing to systolic hypertension, increased PP, LVH, diastolic dysfunction, stroke, atherosclerotic coronary and peripheral disease, congestive heart failure, and myocardial infarction [27, 68]. Although arterial stiffness and structural remodeling is as a natural consequence of aging, it occurs at an accelerated rate in individuals who suffer from the MetS [129, 130].

The purpose of this study was to examine the impact of a therapeutic intervention, of aerobic exercise training, on arterial stiffness and wall remodeling in individuals with the MetS, compared to inactive MetS participants. We evaluated the effect of aerobic exercise training on carotid IMT, arterial remodeling that occurs as a consequence of stiffening. We also observed the effect of aerobic exercise on PWV and cPP, both measures of arterial stiffness. No previous study had examined the effect of chronic, aerobic exercise on measures of stiffness or wall thickness in MetS individuals. The major finding of this study was that 8-weeks of aerobic exercise training significantly attenuated measures of arterial stiffness in MetS individuals, but did not have an effect on carotid IMT.

## 5.1. Summary of Study Participant Characteristics

Prior to examining the aims of the thesis, it was important to first analyze the baseline characteristics of MetS individuals compared to healthy controls. The presence of the Mets, defined using the NCEP ATP III [55] definition, was clearly demonstrated in the baseline metabolic and hemodynamic characteristics (table 4.1). The Mets group had triglyceride parameters 75% higher, HDL cholesterol 16.5% lower, and BP 16% higher than healthy controls. Increased waist circumference is considered a key inclusion criterion for the MetS. In fact, 86% of our study population met the waist circumference inclusion criteria, and the average circumference was 39% higher than those without the syndrome. Recently, there has been an increased focus on the accumulation of excess fat in the abdominal region, and it has been proposed that waist circumference is a better indicator of the risk of developing CVD, than either BMI or the waist-to-hip ratio [9]. Also, a pattern of abdominal obesity correlates more strongly with insulin resistance, a major risk factor for diabetes, and can cause arterial stiffness by disrupting the NO-VSMC pathway, increasing VSMC tone [131]. Further, we performed regression analysis comparing the MetS inclusion criteria to the MRS. All indices were strongly correlated with the MRS (table 4.3), with even distribution, except for lower, negative correlation of HDL cholesterol to the MRS.

The present study demonstrated that people with the MetS had increased stiffening, measured as PWVcf, compared to their healthy counterparts (figure 4.1). This is in agreement with previous studies showing accelerated arterial

stiffening in MetS individuals compared to healthy controls [130]. However, the MetS was shown to be unrelated to upper limb PWV, measured between the carotid and radial sites. The control group on average had a PWV<sub>cr</sub> of 7.9 m/s and the MetS 8.0 m/s. Regression analysis also confirmed that there is no relationship in peripheral measures of arterial stiffness to the MRS, but both PWV<sub>cf</sub> (figure 4.12) and cPP (figure 4.14) had a positive correlation to the MRS. This confirms previous findings that various arterial segments respond differently to arterial remodeling [73]. In a healthy arterial system, the compliance of arteries decreases with increasing distance from the heart, and the velocity of the wave travels faster in more muscular, peripheral arteries as elastin material declines [132]. Central, elastic arteries lose compliance with advancing age and increased metabolic risk, whereas compliance in the peripheral arteries change little with advancing age and disease states [133, 134]. This results in an inability of the central arteries to cushion pulse waves generated from the heart, placing increased pressure on the microcirculation and earlier return of the wave, increasing LV workload [75].

Previous investigations suggest factors such as age, HR, and height are key factors that influence central and peripheral pressures, and can therefore modulate PWA and PWV [47]. In a stiffened artery, the increased duration of ejection period and slowed HR, as a result of LVH, increases the likelihood that the reflected wave will return earlier during systole, reducing the boost to coronary perfusion [24]. Small statures leads to an earlier return of the wave because points of reflection are closer to the aorta. Conversely, with increased

height the reflection sites are farther from the aorta, and the reflected wave arrives at a later point, during diastole, reducing the pressure load on the LV and increasing myocardial perfusion [24]. Therefore, an individual of short stature and a higher workload, as a result of a slower HR, could suffer from a higher PWV, that is not dependent on mechanical stiffening. In our study there was no significant difference in HR, height, or age (table 4.1) between the groups, therefore our results are not confounded by these characteristics.

Individuals with the MetS also had a marginally higher SBP than patients without the syndrome, and BP is both a well-known cause and consequence of large artery stiffness [135]. At higher BP, the elastic modules of collagen dominate and the non-linear nature of elasticity should be underlined: the change in elastic modulus for a given change in intra-arterial pressure is greater at high BP than at low BP [135]. Thus, BP increases can lead to a significant PWV enhancement. This decrease in compliance, with increasing BP, should be distinguished from structural changes leading to increased stiffness [47]. This supports the notion that the increased stiffness, observed in the MetS population, is not a sole consequence of the increase in distending pressures.

An interesting finding of this study is that the MetS individuals had a cPP 10 mmHg higher than the healthy controls, with no significant difference in bPP (figure 4.2). There are several limitations that exist with peripheral BPs, that can be reduced with the accurate measurement of cPP [24]. Historically, DBP had been regarded as the primary indicator of CV risk. However, it ignores the pulsatile component (PP) of arterial pressure [135]. The disparity between central

and peripheral PPs are mainly characterized by the differences in arterial stiffness and wave reflection [136]. In our population of MetS individuals the increased reflections and stiffness were related with increased central pressures independently of brachial pressure. McEniery et al [136] confirmed CV risk factors such as hypertension, smoking, diabetes, and high cholesterol were all associated with more profound increases in central pressures, even after adjusting for differences in age, height, and HR. Central pressures are now considered to be a more accurate predictor of CV events [137, 138], react differently to hypertensive medications [139], and are a better representation of pressures in the central arteries because of pressure amplification [136]. Recently, Miyashita et al. [139] confirmed that antihypertensive drugs with vasodilator properties lower central SBP independently of peripheral BP, whereas non-vasodilators may even raise central SBP.

In a healthy, compliant arterial system, there is a gradual widening of PP moving from the central to the peripheral arteries as SBP rises with increasing distance from the heart and DBP declines slightly [47]. In a stiffened artery the amplification of pressures is attenuated as cPP increases [136]. Indeed, our analysis showed a positive correlation of PWVcf to cPP (figure 4.17), but no correlation with bPP. This supported past findings, suggesting cPPs are a better indicator of arterial dysfunction [140]. McEniery et al [136] found considerable inter-individual variability between participants, and by placing individuals in BP categories (normal, stage 1 or 2 hypertensive) overlap occurs between aortic SBP and BP categories. McEniery's group found that greater than 70% of

individuals with high-normal SBP had similar central SBP to those individuals with stage 1 hypertension [136]. There was also significant variation in central pressure between individuals, despite grouping of similar brachial pressures. These are important implications for the categorization of hypertension; if cPP is more important in defining an individual's risk, then categories based on central, rather than peripheral pressure may be more useful.

Our study also found that Aix tended to be 44% higher, when adjusted for HR, in the MetS individuals compared to healthy controls (figure 4.4). There is evidence that increased arterial stiffness can cause a more rapid return of the reflected systolic wave from small peripheral vessels, such that this augments central aortic systolic pressure [141]. This would increase cardiac afterload and reduce diastolic pressure and coronary perfusion. Although Aix failed to reach significance, our data clearly demonstrated elevated arterial stiffness in the Mets group, through PWV and cPP.

Arterial stiffness is influenced by structural components related to the architecture and composition of the arterial wall, such as the thickness of the intima and media, and the type and content of collagen and elastin in the extracellular matrix [96]. As presented earlier, IMT occurs with aging, even in populations without incidence of atherosclerosis [81]. A thickened carotid artery is relevant in the discussion of stiffening because of the changes that occur in the arterial wall composition in a stiffened artery. Increased stiffness contributes to a greater rise in cPP, which is a strong determinant of CV events, including CAD and stroke [71]. Remodeling of the carotid artery may be a large contributor to

CVD, and the association of carotid IMT with CAD and stroke may thus involve a common mechanical factor, such as cPP. We confirmed that PWVcf was positively correlated with both IMTmax and IMTavg (figures 4.15) and that cPP was strongly correlated with both measurements of carotid IMT (figure 4.16). Boutouyrie et al [71] also found a stronger relation of carotid pulse pressure ( $r=0.42$ ,  $P<0.001$ ) than brachial pulse pressure ( $r=0.27$ ,  $P<0.001$ ) to carotid IMT in 167 normotensive and hypertensive volunteers. The rise in central pressures observed in the MetS group, could be responsible for some of the carotid thickening, in response to the increased fatiguing effect of cyclic stress observed in elevated pressures, which results in hyperplasia and hypertrophy of VSMC and increased collagen deposition [69].

Carotid IMT measured at both the average and maximum point were found to be higher in the MetS group compared to controls (figure 4.3), although maximum IMT barely failed to reach significance. Carotid IMTavg was 0.07 mm higher and carotid IMTmax 0.11 mm higher than the control group. The future risk of myocardial infarction increases by 15%, and stroke risk by 18%, with an increase of 0.1 mm in carotid IMT [56]. We also confirmed that both IMTavg and IMTmax were positively correlated with higher metabolic risk (figure 4.13).

Dynamic factors are mainly related to VSMCs and regulated by paracrine, endocrine, and neural mechanisms, including endothelium derived compounds [141]. Endothelium-derived compounds, such as NO, endothelium-derived hyperpolarizing factors, prostaglandins, and endothelins, play a major role in the dynamic regulation of arterial tone, and appear to modulate the state of arterial



stiffness [141]. Several lines of evidence support an association between arterial stiffness and endothelial function in humans, and brachial artery endothelial dysfunction also correlated with increased carotid IMT [93, 140]. There is also evidence for the reverse association; the inability of endothelial cells to express NO synthase while in stiffened arteries is substantially reduced [94]. Therefore, it is possible that endothelial dysfunction could contribute to the early stages of arterial stiffness, which could then further aggravate endothelial dysfunction in a vicious cycle.

We examined endothelium-dependent vasodilation, using the FMD technique. We discovered no significant difference in endothelial function, under basal conditions, between the MetS individuals and healthy controls (figure 4.6). However, % change in FMDflow, when normalized to FMD:SR<sub>AUC</sub>, was 30% lower in the MetS group, compared to the healthy controls. A greater effect was observed in a type II diabetic population, that had significantly lower endothelial-dependent vasodilation, compared to healthy controls [90]. This could be attributed to the more advanced stage of disease in this group. When compared to the MetS group, the diabetic population could have more severe insulin resistance and structural alterations of the arterial wall. Hyperglycemia can contribute to defective insulin-stimulated vasodilator capacity through increased AGEs, formation of oxygen-derived free radicals, and disruption of the NO-VSMC vasodilator pathway [90]. Also, high total cholesterol (which may be significantly more advanced in type II diabetics), not measured in our study, is associated with endothelial dysfunction [90].

## 5.2. Effects of Training on Measures of Arterial Stiffness

In the present study we found strong evidence that arterial stiffness decreases with aerobic exercise in MetS individuals, compared to their MetS counterparts who remained sedentary. This was observed through a significant decrease of almost 1 m/s in PWVcf (figure 4.7) and a tendency for Alx (figure 4.9) to decrease in the trained group. The 11% reduction in PWV and 20% decrease in Alx@75 are correlated with a significant decline in CV risk; a 1 m/s decrease in PWV is equivalent to a 14% reduction in risk [7]. There was no significant difference in HR, height, BP, or age (table 4.2) between the groups.

Although the mechanisms underlying the association between aerobic exercise training and arterial stiffness are not fully understood, there are several possible explanations. There is strong evidence that the improvement is not linked to traditional risk factors, as there was no significant change in BMI or body fat %, hemodynamic, or metabolic parameters after the exercise intervention (table 4.2). This suggests aerobic exercise is related to an improvement in arterial function, regardless of changes in metabolic risk. A major finding of this study was that the decrease in stiffness occurred with an increase in peak aerobic capacity, in the trained group. Cross-sectional analysis revealed that the MetS group had a significantly lower  $VO_{2peak}$  compared to the control group (figure 4.5). We also showed that  $VO_{2peak}$  was negatively associated with the MRS (figure 4.18). In relation to CV risk, Jae's group found that men with the MetS who maintain high cardiorespiratory fitness levels have a lower risk of mortality

than unfit men without MetS [111]. It has also been shown that low aerobic capacity (measured as MET) is a stronger predictor of CVD and mortality than other established risk factors, such as hypertension, smoking, and diabetes [58]. The same group found that with decreasing fitness, the relative risk of death from all causes nearly doubled in all subgroups possessing a CV risk factor (hypertension, COPD, diabetes, smoking, high cholesterol, obesity).

Lower arterial stiffness is correlated with higher levels of fitness in MetS individuals who have participated in life-long, more consistent, pursuit of exercise [111]. However, it was not known whether previously sedentary, MetS individuals could benefit from implementing a regular exercise regime into their lifestyle. Therefore, the present study provided novel evidence that participating in aerobic exercise effectively reduced arterial stiffness, while improving peak aerobic capacity.  $VO_{2peak}$  improved by 14% in the trained group, while the non-trained group had no change (figure 4.11). We also supported previous studies [37] that composite measures of stiffness were inversely correlated with peak aerobic capacity, measured as  $VO_{2peak}$  (figure 4.19).

The link between the improvement in  $VO_{2peak}$  and attenuation of stiffness could be due to a number of potential mechanisms, described further later in the discussion. Briefly, aerobic exercise decreases inflammation and oxidative stress, both elevated in the MetS, which contribute to increased arterial stiffness [115]. Exercise also has been shown to improve insulin sensitivity, improving arterial compliance and endothelial-dependent vasodilation with an increase in NO bioavailability [38]. Aerobic exercise can attenuate excessive sympathetic

activity, decreasing arterial tone, improving compliance in a relatively short period of time [112]. Finally, it is possible that the elastin-collagen composition changed. The increase in pressures and mechanical distension during exercise bouts can stretch collagen fibers and modify their cross-linking [112]. Although it is believed that structural changes occur more gradually over a longer period of time.

Conversely, it is possible that the increased arterial stiffening in individuals with the MetS may have directly caused the decrease in aerobic capacity. An increase in stiffness contributes substantially to the workload of the LV. Ejection of blood into a stiff aorta, coupled with early return of reflected pressure waves, increases cPP and cardiac energetic demand, which reduces myocardial oxygen supply and consumption, decreasing coronary perfusion [112]. Therefore the changes in fitness levels may be due to a decrease in stiffness and improvement in the ventricular-arterial relationship, rather than the converse relationship.

An attenuation of endothelial dysfunction, and consequent decrease in VSMC tone may explain some of the reversal in stiffness after aerobic exercise training. The contribution of VSMCs to large artery stiffness is proportional to their level of active tone and effect arterial stiffness through their regulatory influence on distending pressure as well as by affecting the physical properties of various arterial wall components [4]. In a healthy endothelium, cells synthesize NO, which then diffuses to VSMCs causing an intracellular cascade of events. This leads to a decrease in tone and an increase in vasodilator capacity of the artery. Shear stress was determined to be a principal physiological stimulus to arterial adaptations associated with exercise training [43]. Tinken et al [108] placed a cuff

around one arm, during training sessions, to produce a unilateral decrease in the blood flow and shear stress associated with exercise bouts. The group found that despite similar effects on forearm volume, girth, and strength, vasodilator function, measured as FMD % diameter change, improved only in the limb exposed to increases in shear stress [108]. Shear stress is an important stimulus that provokes the increase in NO release, and the consequent increase in vasodilator response as VSMC tone decreases. Indeed our group found that FMD did improve (figure 4.10) after normalizing to shear rate in the trained group, falling short of reaching significance. However, the shear stress provoked during the aerobic exercise bouts (not measured), could be responsible for some of the decrease in stiffness observed in our population.

Endothelium-dependent vasodilation declines with an increase in the MRS and is increased in Mets individuals [142]. Our data did not show any significant improvement in endothelial function dependent on flow, measured with FMD, in the MetS trained individuals (figure 4.10). However, there was a slight improvement of change in post-stimulus diameter as a percentage of the baseline diameter, which is considered to be a more robust assessment of endothelial-dependent vasodilation. Although, the 21% improvement was not significant, a previous study, utilizing computer-assisted FMD analysis, did show a significant improvement in FMD after acute and chronic exercise in the MetS population [16]. Normalization of FMD to  $SR_{AUC}$  has recently been proposed to control for the presence of the large inter-subject variability in reactive hyperemia-induced shear stress [143]. We observed an increase of 43% in the

FMD response, when normalized to  $SR_{AUC}$ . One reason that could explain why our subjects did not show a significant improvement in endothelial function could be due to the large degree of observer error associated with manual assessment of arterial diameters, using visual inspection of single frames [125]. Once the arterial image for analysis is chosen, the boundaries for diameter measurements (the lumen-intima interfaces) are identified manually with electronic calipers or automatically using edge-detection software. Computer-assisted analysis, utilizing edge-detected and wall-tracking software, has demonstrated significantly lower intra-observer variation compared to the manual technique [125]. In addition, a study done in type II diabetics showed an improved endothelial-dependent vasodilation response, following exercise, compared to no change for healthy controls, who also participated in the exercise protocol [38]. A possible explanation may be related to an improved insulin sensitivity or change in total cholesterol. Therefore, the lack of significant change of FMD in our MetS group, could also be explained by the less advanced state of disease and insulin insensitivity, compared to type II diabetics.

VSMC cell tone is also influenced by sympathetic nervous system activity [85]. Increased sympathetic activity and elevated levels of vasoconstrictor circulating factors (norepinephrine, epinephrine) are associated with depressed levels of NO, reducing endothelial function and increasing stiffness [144]. Also, both stiffer arteries and endothelial dysfunction play a role in MetS risk factors that contribute to baroreflex dysfunction, which tends to reinforce sympathetic outflow and arterial distensibility; and potentially contribute to increased tone [144]. All of

these factors together contribute to a vicious cycle of high sympathetic outflow, reduced vasodilator function and increased CVD risk. Reduced HR variability, a measure of autonomic balance, was associated with an increase incidence of CAD, myocardial infarction, and fatal heart disease and total mortality in a diabetic population [144].

There is strong evidence to suggest that exercise training can keep the autonomic nervous system healthy, and arterial compliance can be altered in a short period of time with exercise, via modulation of sympathetic influence of VSMCs [112]. In both middle-aged and older subjects the baroreflex function was worse in most sedentary subjects compared to those who participate in moderate exercise, and better yet in those who perform regular endurance exercise [144]. Thijssen et al [145] showed that acute changes in arterial tone altered both femoral and carotid IMT after acute administration of glyceryl trinitrate, a NO donor that improves vasodilation through VSMC relaxation. This suggests that changes in sympathetic activity may contribute to changes in arterial wall thickness. However, it is currently unknown whether changes in autonomic balance and IMT as a result of chronic exercise training are correlated. Unfortunately, sympathetic activity was not measured in our study; therefore we can only theorize that the same influence of VSMC on elasticity occurred.

It is not known if short-term exercise interventions alter the structural composition in the arterial wall. However, it is possible that the increased shear stress and mechanical distension during exercise modify collagen fiber cross-linking, thereby arterial elasticity [112]. As explained earlier, changes in large artery

compliance following an aerobic exercise intervention are relatively rapid and may be more heavily impacted by functional change. The increase in stiffness, and rise in cPP, increases the risk of carotid thickening, in response to insults via hyperplasia and hypertrophy of VSMC and increased collagen deposition [69]. We showed that composite measures of stiffness were strongly correlated with both IMTavg and IMTmax (figure 4.15 and 4.16). Following repetitive systolic stretching, the elastic lamellae become more disorganized, collagen content increases, VSMC hypertrophy and AGEs form degrading cross-links with elastin content, contributing to the increase in thickening [146]. This process occurs with age, and is often accelerated in a diseased state. Our study discovered no change in carotid IMT after 8 weeks of aerobic exercise training (figure 4.8).

Previous studies have proven to be inconclusive on whether an exercise intervention can attenuate IMT in either the carotid or peripheral sites. Thijssen et al [2] found a significant decrease in brachial wall thickness following 8 weeks of handgrip exercise. Another group revealed a significantly lower superficial femoral, brachial, and most relevant, carotid artery wall thickness in squash athletes compared to sedentary, healthy controls [44]. Tanaka et al [45] showed an increase in carotid IMT with increasing age, in both sedentary and endurance-trained healthy men. Within the same group, 18 healthy middle-aged and older sedentary subjects participated in 3 months of aerobic exercise (4-6 days a week for 45 minutes) and saw no change in carotid IMT [45]. Other studies reported an inverse relationship between  $VO_{2peak}$  and carotid IMT [147, 148]. Therefore, it was our hypothesis that exercise intervention in a diseased population that



experiences more advanced carotid IMT, would show some attenuation of wall remodeling. We believe the lack of attenuation in distending pressure may be linked to the IMT observations. Both cPP (figure 4.16) and cSBP showed a strong relationship with average and maximum carotid IMT. This was also confirmed in a previous study of a healthy population [45]. The absence of reductions in carotid IMT with exercise intervention was accompanied by a corresponding absence of reduction in carotid SBP and PP (table 4.2). Future aerobic interventions, with increased sessions of a prolonged nature, may provide different results of action on central pressure indices and arterial wall remodeling in the MetS.

Several other mechanisms are probably involved and require further investigation; we attempt to enumerate upon them, without an in-depth discussion. These may include alterations in other vasodilator factors (prostaglandins, hyperpolarizing factor), vasoconstrictor molecules (endothelin-1 and angiotensin II), inflammatory mediators, reactive oxygen species and antioxidant defenses; which can all influence VSMC tone and remodeling [142]. In addition to the improved endothelial dysfunction observed after aerobic exercise, Tjonna et al [16] also showed a reduction in oxidized LDL, which directly influences NO bioavailability, and a significant increase in adiponectin. High fitness is associated with low levels of MetS risk factors, abdominal fat, insulin resistance, inflammation, oxidative stress, and BP all of which are independently associated with increased arterial stiffness [111]. Both insulin resistance and inflammation have been shown to be associated with endothelial dysfunction and

wall thickening, which may increase arterial stiffening [43]. BP was not improved in our group, and insulin resistance or inflammation was not measured.

We also theorize that a decrease in insulin sensitivity and improved fatty acid oxidation, taken together with reduced plasma oxidized LDL levels, could result in a positive influence on arterial function. Adipocytes, in particular from visceral abdominal regions, produce several bioactive peptides, such as angiotensin, interleukin-6, plasminogen activator inhibitor-1, leptin and adiponectin, which in turn impact on arterial structure and function [149]. Elevated leptin and low adiponectin levels are commonly found in association with the MetS and have been associated with endothelial dysfunction and arterial stiffening in the MetS [149]. Conversely, adiponectin stimulates NO production, reducing expression of adhesion molecules and cytokine production [149]. Adiponectin levels increase with exercise, inhibiting markers of inflammation, improving arterial compliance [149]. As presented earlier, 86% of our MetS population met the waist circumference inclusion criteria and on average had an obese BMI of 37 (table 4.1). This increase in abdominal obesity could correlate with an increase in leptin and decrease in adiponectin. In addition, metabolic toxins, such as free fatty acids and inflammatory cytokines, including interleukin-6 and tumor necrosis factor-alpha, might alter endothelial signaling pathways, such as the PI3K–Akt pathway, increasing VSMC tone [84]. Future interventions incorporating the use of biomarker assessment, in relation to arterial stiffness, could discover some of the before mentioned possibilities of mechanistic changes.

## **1.4. Limitations of the Study**

Because our exercise intervention was completed by a relatively small number of subjects, the benefits of aerobic training on arterial stiffness need to be confirmed by larger studies. The fact that a short 8-week intervention produced a sizable decrease in arterial stiffness suggests that larger exercise studies, of longer duration, in this high-risk group may attenuate measures of arterial remodeling.

The method used to measure FMD was prone to a larger degree of observer error compared to the computerized diagnostic program. Future use of the computerized method could improve the endothelial-dependent response reliability. In addition, shear rate measured during an acute bout of exercise could determine the role on improved endothelial function.

Neurohumoral, inflammatory, oxidative, and metabolic biomarkers can directly influence arterial stiffness and wall remodeling. However, the improvements observed in the MetS trained group cannot be pinpointed to a specific mechanism and future studies should therefore incorporate some of these procedures.

Finally, the time-dependent relation between increased cPP and arterial remodeling could not be determined in the present study because of its cross-sectional design.

# **Chapter 6**

## **Conclusions**

The purpose of this thesis was to examine the impact of aerobic exercise training on arterial stiffness and wall remodeling in individuals with the MetS. Arterial dysfunction, due in part to arterial stiffening, is recognized as a surrogate end point for CVD and predicts the risk of future CV events. The MetS is a clustering of risk factors associated with increased stiffening and carotid artery thickening. Aerobic exercise is a well-established therapy that reduces CV morbidity and mortality and has proven to be an effective intervention against arterial stiffening and pathological wall remodeling in healthy, previously sedentary, individuals and other diseased populations [12]. However, no study had examined the effect of chronic aerobic exercise on arterial stiffness or remodeling in MetS individuals.

In our study, the PWVcf and cPP were 26% and 33% higher, respectively, in MetS individuals compared to controls. Carotid IMT was 13% greater in the MetS group. The major finding of this study was that 8 weeks of aerobic exercise significantly attenuated measures of arterial stiffness in the trained MetS individuals, but did not have an effect on carotid IMT. Another important discovery was the 14% improvement in  $VO_{2peak}$  in the trained population, compared to no change for the non-trained group.

The results described in this thesis have progressed the understanding of aerobic exercise as an effective therapeutic intervention for the arterial stiffening observed in the MetS. Although it was out of our scope to directly measure the mechanistic changes that are responsible for the improvement in arterial compliance, our data supports the theory that the changes associated with an improvement in peak aerobic capacity may facilitate these developments.

# **Chapter 7**

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