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Defining Cardiovascular Dysfunction in the Metabolic Syndrome: therapeutic and nutritional approaches to treatment

Sara Brooks Fournier

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Defining Cardiovascular Dysfunction in the Metabolic Syndrome: therapeutic and nutritional approaches to treatment

Sara Brooks Fournier

Dissertation submitted to the
School of Medicine at West Virginia University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Exercise Physiology

Paul D. Chantler, Ph.D., Chair

Taura L. Barr, RN, Ph.D.

Gregory M. Dick, Ph.D.

Jefferson C. Frisbee, Ph.D.

I. Mark Olfert, Ph.D.

Division of Exercise Physiology

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Abstract

Defining Cardiovascular Dysfunction in the Metabolic Syndrome: therapeutic and nutritional approaches to treatment

Sara Brooks Fournier

Metabolic syndrome (MetS) is a complex disease state defined by the manifestation of a cluster of cardiovascular (CV) risk factors including, abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. Individuals afflicted with MetS have been demonstrated to assume the burden of a 3-fold increased risk of cardiovascular disease (CVD) mortality, myocardial infarction, stroke, type II diabetes (T2DM) and all cause mortality. The national prevalence of hyperglycemia and abdominal obesity paralleled by a surge in sedentary lifestyle behavior underscore the importance of early identification and treatment of MetS. Deleterious adaptations to both vascular and myocardial structure and function have been demonstrated in MetS. Alterations in CV function associated with accelerated arterial aging have been implicated in the association between MetS and CVD. The underlying pathophysiology of MetS is not well understood and CV dysfunction has not been comprehensively examined in a MetS population free from confounding pathologies including T2DM and/or overt CVD. Furthermore, the effects of therapeutic lifestyle interventions for targeting subclinical CV dysfunction in MetS without T2DM and/or overt CVD require investigation. Therefore, the directive of the studies included in this dissertation was to perform a comprehensive assessment of CV function to validate the presence of CV dysfunction in MetS and to evaluate practical therapeutic strategies for improving cardiac and arterial dysfunction associated with MetS. Results from these studies identified subclinical left ventricular (LV) diastolic dysfunction at rest LV systolic dysfunction during exercise. Additionally, it was discovered that deleterious adaptations to large artery structure and function occur in individuals with MetS. Importantly, these alterations occurred in the absence of chronic disease including T2DM and clinical CVD. These results suggest for the first time that CV dysfunction does occur in individuals afflicted with MetS without T2DM and/or CVD. The investigation of exercise training as a therapeutic strategy for targeting arterial dysfunction revealed improvements in central arterial stiffness, central systolic blood pressure and aerobic capacity after 8 weeks of moderate/high intensity aerobic training. During peak exercise we have demonstrated improvements in arterial-ventricular coupling, LV contractility, peripheral vascular resistance, and aerobic capacity after 8 weeks of aerobic training in MetS. Exercise training was unable, however, to improve resting LV structure, LV diastolic function, or metabolic profile. Results from our investigation using a nutritional therapeutic approach to CV dysfunction in MetS demonstrate similar inconclusive findings. Single-dose supplementation with two doses of *trans*-resveratrol demonstrated no conclusive therapeutic potential for improving arterial stiffness or reducing central systolic blood pressure. These results would suggest that a greater understanding of the underlying pathology of

MetS is required for identifying and optimizing therapeutic approaches to its treatment. Taken together the results of these collective documents demonstrate an understanding of the synergistic effects of co-occurring risk factors on CV function in the absence of chronic clinical disease. Further, these results highlight therapeutic and nutritional strategies for targeting CV dysfunction in MetS.

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List of Abbreviations

Angiotensin II	Angiotensin II
AGEs	Advanced Glycation End Products
AHA	American Heart Association
AS	Arterial Stiffness
AT	Aerobic Training
BH₄	Tetrahydrobiopterin
BMI	Body Mass Index
cGMP	cyclic guanosine monophosphate
CHD	Coronary Heart Disease
COX1	Cyclooxygenase 1
COX2	Cyclooxygenase 2
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DT	Deceleration Time
Ea	Arterial Elastance
Ea/Ees	Arterial-Ventricular Coupling
ECs	Endothelial Cells
ED	Endothelial Dysfunction
EDV	End Diastolic Volume
Ees	End-Systolic Elastance
EF	Ejection Fraction
eNOS	Endothelial Nitric Oxide Synthase
ESP	End Systolic Pressure
ESPVR	End-Systolic Pressure-Volume Relation
ESV	End Systolic Volume
GLUT4	Glucose Transporter Type 4
GTP	guanosine-5'-triphosphate
HDL-C	High-Density Lipoprotein Cholesterol

HOMA-IR	Homeostatic Model Assessment-Insulin Resistance
HR	Heart Rate
HTN	Hypertension
IVRT	Isovolumic Relaxation Time
LDL-C	Low-Density Lipoprotein Cholesterol
LVH	Left Ventricular Hypertrophy
MetS	Metabolic Syndrome
MMPs	Matrix Metalloproteinases
NCEP ATP III	National Cholesterol Education Program ATP III
NHANES	National Health and Nutrition Examination Survey
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
PGH₂	Prostaglandin H ₂
PGI₂	Prostacyclin
PRSW_i	Pre-load Recrutable Stroke Work
PVR	Peripheral Vascular Resistance
ROS	Reactive Oxygen Species
RT	Resistance Training
SBP	Systolic Blood Pressure
SEVR	Sub-Endocardial Viability Ratio
SNS	Sympathetic Nervous System
SV	Stroke Volume
SW	Stroke Work
T2DM	Type II Diabetes Mellitus
TAC	Total Arterial Compliance
TG	Triglycerides
TIMPs	Tissue Inhibitor of Metalloproteinases
TxA₂	Thromboxane A ₂
VSMC	Vascular Smooth Muscle Cell
WC	Waist Circumference

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Chapter 1

Literature Review

Metabolic Syndrome

In 1988 Gerald M. Reaven established “Syndrome X” to identify high-risk patients with co-occurring metabolic risk factors predisposed to cardiovascular disease (CVD) and type II diabetes (T2DM). Today we refer to the manifestation of a cluster of co-morbidities as the metabolic syndrome (MetS). MetS as defined by the guidelines set forth by the US National Cholesterol Education Program Adult Treatment Panel III (NCEP) requires the manifestation of at least three of the following five risk factors: abdominal obesity, dyslipidemia (e.g. increased triglycerides, and decreased high density lipoprotein), hypertension, and hyperglycemia. The prevalence of MetS among U.S. adults (≥ 20 years of age) estimated from data collected using the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2010 indicates a decreasing trend over the last decade from 25.5 percent to 22.9 percent [1]. The source of the descending shift in MetS reflects individual decreases in the prevalence of hypertriglyceridemia and in elevated blood pressure, both of which are correlated with increases in the use of anti-hypertensive and lipid-modifying drugs. Although this trend appears to reflect a favorable shift, the increasing trends in the prevalence of hyperglycemia and elevated waist circumference (abdominal obesity) and predictions based on the spread of the American obesity epidemic estimate that the future incidence of MetS in the US will continue to rise [2]. The American epidemic of obesity paralleled by a surge in sedentary lifestyle behavior underscore the importance of early identification, treatment, and

prevention of MetS. Compared to healthy individuals, those who present with MetS assume the burden of a 3-fold increased risk of cardiovascular disease (CVD) mortality, myocardial infarction, stroke, and all-cause mortality [3-5] and T2DM. The pathophysiological mechanisms underlying these associations are incompletely understood, however. Existing literature has reported evidence of both vascular and myocardial abnormalities in MetS. Indeed our lab and others have shown that MetS is associated with the acceleration of arterial aging. Grouping of metabolic risk factors can also affect functional properties of the arterial system, promoting vascular endothelial dysfunction. Given that large elastic artery stiffness (AS) is a feature of MetS [6-7] and impaired endothelium-dependent dilation of the brachial artery has been identified in the presence of MetS, pathophysiological changes to the arterial vasculature may represent an important link between MetS and increased CVD risk.

Macrovascular Adaptations

The large elastic arteries serve two important functions. First, they act as conduits to deliver oxygen-rich blood pumped from the left ventricle (LV) to peripheral arteries. Second, large distensible conduits (e.g. aorta) are responsible for the Windkessel effect, which helps to maintain a relatively constant arterial pressure despite the pulsatile nature of blood flow from the LV. During systole the elastic aorta expands to store a portion of the blood ejected from the LV. During diastole, potential energy created from the expansion and subsequent passive elastic recoil of the aorta helps to maintain blood pressure

and provide continuous steady blood flow to peripheral vessels. With aging and under pathological conditions, the Windkessel effect is diminished as a result of increases in AS and a loss of large elastic artery compliance [8].

Conduit Remodeling

The large elastic arteries are comprised of three distinct structural layers: the tunica adventitia, the tunica media, and the tunica intima. Collagen and elastin filaments responsible for the elastic properties of large arteries reside within the tunica media, which is largely made up of smooth muscle cells and the extracellular matrix. Biomechanical properties (stiffness and viscoelasticity) of the aorta are determined primarily by the elastic matrix, which consists of elastic fibers, collagens, proteoglycans, and glycoaminoglycans. Changes to conduit artery structure can occur with normative aging as well as in the presence of pathological stimuli in an effort to maintain arterial wall integrity. In individuals with MetS, changes occur to the structure and function of the arterial system that compromise the efficiency of the cardiovascular system and increase the risk for the development of CVD. Two key contributors to arterial dysfunction associated with MetS include arterial remodeling and large elastic artery stiffness (AS).

Arterial remodeling describes changes in vessel size; more specifically this term refers to changes in the cross-sectional area within the elastic lamina surrounding the vessel. Existing studies in healthy humans and nonhuman primate and rodent models of aging provide evidence of luminal dilation in the central elastic arteries with advancing age, leading to an increase in lumen size

[9]. In healthy humans, outward remodeling of elastic arteries is a homeostatic response to changes in blood flow in order to maintain optimal levels of shear stress and circumferential wall tension [10].

MetS is regularly associated with thickening of the carotid wall, indexed as carotid intimal medial thickness (cIMT). IMT refers to the measurement of the thickness of the innermost layers of the arterial wall: the tunica intima and tunica media. IMT is most commonly used in clinical trials and in observational studies as an important endpoint for the evaluation of the progression or the regression of atherosclerotic CVD [11]. Although IMT is strongly associated with the identification of subclinical atherosclerosis, factors other than plaque formation have an influence on IMT. Indeed, IMT has been shown to increase linearly as much as 3-fold between the ages of 20 and 90 years in the absence of atherosclerosis [12]. Further, studies using animal models not known to develop atherosclerosis, including rodents and nonhuman primates, report thickening of the intima media with aging [13-14]. In MetS cIMT has been shown to increase between 0.08 mm and 0.11 mm vs. healthy controls [7, 15]. Specifically, when considering impact of specific clusters of MetS components, the presence of low HDL and abdominal obesity increases the probability of having an extreme common carotid artery (CCA) IMT (i.e. values >2 SD from the mean of CCA IMT) by 70 percent compared to those without reduced HDL or abdominal obesity [7]. A recent longitudinal study examining the temporal relationship between structural arterial remodeling and arterial function provides evidence to support the results from cross-sectional studies that suggest maladaptive arterial

remodeling in MetS. Ferreira et al. [16] report that baseline values of interadventitial diameter, lumen diameter (LD), circumferential wall tension (CWT), circumferential wall strain (CWS) but not IMT were significantly elevated in individuals with MetS vs. non-MetS. At follow-up MetS displayed significantly steeper increases in IMT vs. non-MetS (0.011 vs. 0.005 mm/year, respectively), and lumen diameter (0.055 vs. 0.023 mm/year, respectively). Importantly, despite arterial remodeling, changes in CWS tended to decrease over time but remained significantly elevated in MetS compared to non-MetS. These findings suggest that arterial thickening in MetS occurs in advance of changes in CWS to restore arterial wall integrity. Interestingly, those individuals who recovered from MetS at the 6-year follow-up and non-MetS displayed similar arterial properties, pointing to the reversible nature of an early pathological process.

Large Elastic Artery Stiffening

In addition to changes in arterial structure the large elastic arteries (i.e. aorta and carotid) are subject to age-related stiffening [17]. Large elastic AS is a strong independent predictor of CVD and mortality. AS has been shown to increase with advancing age [18-19] and in the presence of MetS [15, 20]. Central AS can be assessed non-invasively from carotid-to-femoral pulse wave velocity (PWV). Observational studies in healthy humans have demonstrated a modest increase in central PWV that occurs in midlife [21]. In MetS, increases in AS may have a detrimental impact on cardiac function; Increases in central systolic pressure that accompany AS promote cardiac hypertrophy leading to

decreases in diastolic pressure and impairment of coronary perfusion [22]. For individuals with MetS, the CVD risk associated with increased AS is of particular importance as a 1.0 m/sec increase in AS corresponds to a 15 percent increased risk of CV mortality after adjustment for age, sex and risk factors [23]. Evidence from existing literature indicates that changes in the expression and/or bioactivity of structural proteins in the arterial wall (and heart) lead to AS. AS is influenced by the composition of the extracellular matrix (elastin-collagen network) and by vascular smooth muscle cell function (VSMC) [24-25]. Further, increased collagen production and smooth muscle cell proliferation, as a consequence of endothelial dysfunction, can alter the structure and composition of the arterial wall and increase AS [26-30]. MMPs and TIMPs are key regulators of arterial and cardiac [26-27, 29-30] remodeling. In animal models, increased MMP9 activity, responsible for the degradation of elastin and collagen [31], was directly linked with AS [32] and cardiac stiffness [33]. In hypertensive patients, plasma MMP9 and TIMP1 were elevated vs. controls, and implicated in AS and an increased risk of CV events [34-36]. Notably, TIMP1 inhibits MMP1 activity that increases type 1 collagen (high tensile strength and rigidity) formation. While structural changes that contribute to AS take several months to develop, AS can also be altered acutely by changes in VSMC tone, which is regulated by endothelial cell function and sympathetic nervous system activity [37]. Sympathetic stimulation can alter AS through an indirect change in arterial pressure or through a direct increase in vascular tone, causing vasoconstriction and decreased arterial diameter [38-40]. Moreover, impaired endothelial

dysfunction, as a result of low-grade inflammation [28], enhances vasoconstrictor activity, altering large artery smooth muscle tone [41-43] which may contribute to acute changes in AS.

Large Artery Endothelial Dysfunction

Endothelial dysfunction (ED) is regarded as an early event in the initiation and progression of atherosclerosis, and as a trademark of vascular disease [44-45]. Impaired endothelial function refers to an imbalance in the production of endothelial-derived mediators that regulate vascular permeability, coagulation, platelet aggregation, and vascular tone. Among the specialized functions of the endothelial monolayer in the regulation of vascular homeostasis, its role in controlling vascular tone is considered the most extensively studied and thus, the term ED refers to an imbalance in the relative contribution of endothelium-derived relaxing factors and contracting factors. Under normal physiological conditions, the vascular endothelium produces mediators of vascular hemodynamics in response to physical and chemical stimuli. ECs contribute to the regulation of blood pressure and blood flow through the production and release of vasodilators including nitric oxide (NO) [46] and prostacyclin (PGI₂), as well as vasoconstrictors including endothelin-1, thromboxane A₂ (TxA₂), and angiotensin II (ANG II). The balance between endothelial-derived relaxing factors and contracting factors represents a major determinant of basal vascular smooth muscle tone [47].

In a clinical setting, ED is quantified by an impaired endothelium-dependent relaxation of the vascular smooth muscle in response to a chemical or physical stimulus. Impaired relaxation of the smooth muscle is associated with diminished production or availability of vasodilator substances, particularly NO, due to a decline in the production of NO by the endothelium, inactivation of NO by reactive oxygen species (ROS), or a decline in the availability of co-factors required for the synthesis of NO. In the presence of cardiovascular risk factors (e.g. hypertension, T2DM, hyperglycemia, etc.) the normal production of NO by eNOS (Figure 1) is altered such that eNOS favors the generation of ROS including superoxide, and hydrogen peroxide. This shift in eNOS function is termed “eNOS uncoupling” [48]. EC activation and ED initiate a change in the anti-proliferative, quiescent state of the healthy endothelium to one that promotes increased production of endothelial-derived constricting factors, increased EC permeability, increased expression of adhesion molecules, increased secretion of chemokines, VSMC proliferation, and platelet activation and thrombosis.

Endothelial-dysfunction in conduit arteries is most often quantified by the recording of endothelial-dependent dilation following pharmacological (e.g. nitroglycerin or sodium nitro-prusside) or physiological stimulation of endothelial-mediated NO synthesis [49]. Clinical assessment and identification of ED has been shown to be an independent predictor of cardiovascular events [45]. Impaired endothelium-dependent dilation of the brachial artery has been identified in the presence of MetS, a finding that is expected, as each component of MetS is associated with ED [50-55]. Such findings suggest that ED may

mediate, in part, the mechanisms responsible for the increased CVD risk in MetS. The exact mechanism by which MetS causes ED remains unclear and it is likely that multiple pathways are involved; however, oxidative stress appears to be a primary mechanism contributing to loss of vascular homeostasis in MetS.

MetS is associated with a chronic state of low-grade inflammation [56]. The presence of inflammation and other co-morbidities of MetS including, hyperglycemia and insulin resistance leads to a decreased generation of NO and an increased production of ROS through eNOS uncoupling [48]. Elevated levels of ROS stimulate the continued production of ROS in a positive feedback loop, leading to chronic elevations in oxidative stress and a decrease in NO production, reduced EC sensitivity to NO, and an increase in NO inactivation/degradation [57]. Further, inflammation promotes the release of cell growth factors that contribute to adverse vascular remodeling observed in MetS through stimulation of VSMC proliferation and collagen formation [58].

Cardiac Dysfunction

Existing studies have documented the adverse effects of individual MetS components including hypertension, T2DM, and obesity on cardiac structure and function. In individuals with the MetS, groupings of interrelated risk factors act synergistically to increase the risk of adverse cardiovascular events, such that MetS is associated with a 3-fold increased risk of mortality resulting from CVD, coronary heart disease, and all causes compared to those without MetS [59]. Recent literature has reported evidence of preclinical myocardial abnormalities in

MetS. While it is unsurprising that each component of MetS is known to independently and adversely affect cardiac structure and function, the combination of risk factors has been shown to convey additional risk [60-61]. Consequently, the greater CVD burden associated with MetS may be mediated, in part, by asymptomatic abnormalities in left ventricular (LV) systolic function, which has been noted at rest in some studies [62-65], but not all [66-67].

Left Ventricular Geometry

General agreement exists in regards to adaptations of cardiac structure in the presence of MetS. The impact of MetS on changes in LV mass have been shown to be greater than the contributions of the individual components of MetS (e.g. blood pressure and body size) [68], emphasizing the importance of the synergistic effects of the component of MetS. Indeed, increases in LV mass and relative wall thickness have been reported in hypertensive subjects with MetS compared to hypertensive subjects without MetS [69] indicating an association of MetS with increased LV mass independent of hypertension. Studies comparing LV structure and function between individuals without MetS, pre-MetS, and MetS indicate progressive LV dysfunction and remodeling with an increasing burden of MetS [67]. Similarly, Aijaz et al. [70] identified an increase in LV mass index (91.7 vs. 87.9 g/m²; $P=0.04$) and LV diastolic dysfunction in women (n=1058) with isolated MetS (i.e. excluding individuals with MetS and established hypertension and/or T2DM) compared to those without MetS. LV hypertrophy (LVH) promotes a decrease in coronary flow reserve (decreased capillary density per unit of

myocardium and increased resistance to flow [71]) and is often a precursor of diastolic dysfunction, however, impaired LV relaxation has been identified in MetS patients independent of LV mass [67]. The presence of LVH is a risk factor for CV morbidity and mortality, including the development of systolic and diastolic function and further progression to heart failure [72-73]. Thus, together with impairments in cardiac function, LVH may provide a modest explanation for the increased CV risk in individuals with MetS.

Left Ventricular Function at Rest

Although the LV structural differences in MetS are fairly well agreed upon, consensus is lacking with regard to changes in LV function. The prevalence of diastolic myocardial dysfunction in individuals aged 65 years and older is estimated to be 35 percent in individuals with MetS [74]. In MetS and in T2DM, diastolic dysfunction results from abnormal myocardial active relaxation and an increase in LV passive stiffness due to metabolic derangements and structural remodeling [74]. Previously, Chinali et al. [66] have shown alterations of LV diastolic dysfunction as characterized by early LV relaxation in American Indians. In agreement with this finding, echocardiographic evaluation of parameters related to LV diastolic function including E/A ratio, deceleration time (DT), and isovolumic relaxation time (IVRT) by Turhan et al. [75] provides evidence of early diastolic dysfunction in MetS vs. healthy control subjects. Using cardiac magnetic resonance imaging technology, Nyman et al. assessed cardiac fat compartments and LV function in men with MetS. These authors found that MetS was

associated with LV diastolic dysfunction and LV concentric remodeling. Furthermore, epicardial and pericardial fat correlated with the degree of LV diastolic dysfunction implicating a role for visceral obesity in the development of MetS-associated LV diastolic dysfunction [76].

Some studies [62-64, 66-67, 77], but not all [66-67] have suggested that individuals with MetS present with LV systolic dysfunction at rest. It has been suggested that the elevated risk for CVD in MetS is mediated, in part, by impaired resting LV systolic function; however, conclusions regarding systolic dysfunction in MetS remain controversial with some studies reporting impairments in LV contractile function assessed using a doppler-derived index of myocardial performance [75]. Wong et al. [78] have identified LV systolic dysfunction in patients with MetS using global strain rate, strain, and regional systolic velocity and diastolic velocity. In an effort to explain the broad contrasting results from several human studies it is important to emphasize that many investigators rely on load-dependent measures to assess LV contractility including ejection fraction (EF). Despite its traditional application, EF is a poor prognostic measure of systolic function because it may be potentially influenced by common observations in MetS including loading conditions and chamber remodeling. Other common load-dependent indices used in the literature to evaluate myocardial contractility include fractional shortening, systolic time intervals and strain and strain rate deformation parameters. Using myocardial strain and strain rate imaging to assess intrinsic LV myocardial contractility, Gong et al. reported impaired global systolic strain and strain rate in the septum,

lateral, posterior, anterior, and inferior walls of the LV in patients with MetS. Importantly, subclinical abnormalities in LV systolic and diastolic function were observed in the presence of normal LV EF [79].

Left Ventricular Function during Aerobic Exercise

Exercise testing is a well-established procedure for physiological stress that can be used to elicit cardiovascular abnormalities not present at rest. Pathophysiologic irregularities may emerge when cardiac demands are increased during aerobic exercise. MetS patients often report a reduced quality of life as a consequence of exertional dyspnoea and reduced exercise capacity [74]. The deterioration of exercise capacity in MetS has been suggested to be a consequence of early diastolic myocardial dysfunction [74].

While the assessment of cardiac function in MetS has been documented within the literature, this manuscript will attempt to add to the current understanding of cardiac dysfunction in MetS by addressing some important limitations of existing investigations. A general shortcoming observed in many studies includes the use of load-dependent measures of myocardial contractility. In individuals with MetS, loading conditions including resting heart rate (HR), and chamber geometry are often altered and therefore, may influence load-dependent assessments. Further, the MetS populations considered in the existing literature often include individuals with T2DM and/or overt CVD [67, 78, 80]. With the inclusion of individuals with symptomatic or clinical CVD, observations of CV structure and function may reflect a synergistic consequence

of the presence of MetS and other confounding pathologies rather than MetS alone. Notably, many studies examining CV structure and function in MetS are limited to resting situations when the CV system is attempting to optimize chamber pumping efficiency [81]. During aerobic exercise the CV system prioritizes cardiac efficacy over energetic efficiency to meet the body's increasing demands [82]. Accordingly, maximal graded exercise testing may expose subclinical LV abnormalities undetectable at rest that may predispose this population to CVD.

Arterial-Ventricular Coupling

A comprehensive understanding of cardiac function requires an examination of the interactions between the left ventricle (LV) and the arterial system (arterial-ventricular coupling). Arterial-ventricular coupling (E_a/E_{es}) is a key determinant of cardiovascular function and is most often assessed in the pressure-volume plane as the ratio of effective arterial elastance (E_a ; a lumped parameter of the net arterial impedance to stroke flow [83-84]) to left ventricular end-systolic elastance (E_{es} ; a load-independent index of the contractility and systolic stiffness of the LV [85-86]).

Left Ventricular End-Systolic Elastance

Early work by Suga and Sagawa [87-90] was the first to conceptualized and conceive a role for LV end-systolic elastance (E_{es}) in LV pump function and myocardial energetics. The end-systolic pressure-volume relation (ESPVR) can

be obtained using an excised canine heart preparation to generate a series of pressure-volume loops during acute alterations in preload or afterload at a constant contractile state. Ees is quantified as the slope of the ESPVR and is generally assumed to be linear (Figure 1). Ees represents an index of LV contractility and may be influenced by LV geometric remodeling and the biophysical properties of the myocardium [85-86]. Accordingly, caution must be applied when interpreting the significance of changes in Ees in the absence of abnormalities in other measures of LV systolic function [91]. Ees can be approximated as $[(\text{end-systolic pressure (ESP)} / \text{end-systolic volume (ESV)}) - V_0]$, where V_0 represents the volume-axis intercept of the ESPVR obtained when the ESPVR is extrapolated to zero pressure, assuming a linear relationship. When calculating Ees some assumptions to consider are: 1) that the slope of the ESPVR is linear and is independent of loading conditions; 2) V_0 represents a common intercept with the volume-axis for joining instantaneous pressure-volume points at similar times during the cardiac cycle; 3) Ees is a function of time only, and is independent of instantaneous and past pressure and volume [87-90].

Effective Arterial Elastance

Effective arterial elastance (E_a) is approximated as the ratio of LV end-systolic pressure (ESP) and stroke volume (SV), and is often considered to be a lumped parameter of the net arterial load imposed on the LV [92-93]. Specifically, E_a is an index which integrates elements of arterial load including peripheral

vascular resistance (PVR), total arterial compliance (TAC), characteristic impedance, and systolic and diastolic time intervals [83]. Notably, a significant limitation of E_a is that while it provides information on the steady and pulsatile components of the arterial load, it is unable to provide information on their relative contribution [93]. E_a is determined from pressure-volume loops as the negative slope of the line joining the end-diastolic volume (EDV) and end-systolic pressure (ESP) points (Figure 1). Later work by Kelly et al. [94] showed agreement between E_a measured invasively as ESP/SV from pressure-volume data and E_a calculated from arterial impedance data in normal and hypertensive humans.

Arterial-Ventricular Coupling Ratio

Original efforts by Sunagawa et al. led the way for the conception of innovative methods to compare arterial load and LV performance as traditionally measured in the frequency domain and the time domain respectively [83]. Quantification of effective arterial elastance and LV end-systolic elastance using the same units (mm/mL) provided the first platform for the study of arterial and ventricular interactions. The ratio of E_a/E_{es} is a measure of the interaction between the LV and the arterial system [83]. Early experimental studies discovered that the heart is able to transfer maximal stroke work (SW; mechanical energy) when $E_a/E_{es} = 1.0$ while energetic efficiency (ratio of SW to pressure-volume area) is maximized at lower values of E_a/E_{es} [95]. In healthy men and women, in the resting state, mean $\pm$ SD values of E_a/E_{LV} , E_a , and

E_{LV} measured invasively are $\approx 1.0 \pm 0.36$, 2.2 ± 0.8 mmHg/ml, and 2.3 ± 1.0 mmHg/ml, respectively [96]. Examination of E_a/E_{es} and its components using non-invasive methods offers the potential for a more comprehensive understanding of arterial and LV interactions under normal physiologic conditions and in various disease states.

Arterial-Ventricular Coupling and Its Components During Exercise

At rest, the LV and the arterial system are optimally coupled to produce SW. During exercise, small changes to HR, LV contractility, preload, and afterload ensure that the cardiovascular system adapts to meet the increased metabolic demands of the body. Using adult dogs, Little and Cheng [97] showed that submaximal exercise produced a left-shift in the LV pressure-volume relation with an increased slope (E_{es}) which caused a decrease in the ratio of E_a to E_{es} from rest to submaximal exercise. Notably, despite the decrease in E_a/E_{es} the coupling of the arterial system and the LV remained in the range to produce nearly optimal SW during steady state exercise. Studies in healthy human subjects show agreement with work performed using animal models. For example, Chantler et al. [92] examined E_a/E_{es} at rest and at peak exercise in normotensive and hypertensive subjects and found that E_a/E_{es} decreased in both men and women due to a disproportionate increase in E_{es} vs. E_a during exercise. During exercise the increase in E_a parallels the observed increase in AS despite reduced peripheral vascular resistance [98].

Arterial-Ventricular Coupling in the Metabolic Syndrome

The following section will review the influence of some of the individual components of MetS on arterial-ventricular coupling and its components.

Aging

Normative aging is known to be associated with alterations in LV structure and function in both men and women. Notably, advancing age is accompanied by increases in LV wall thickness and collagen deposition [86]. Consequently, aging is also associated with an increase in resting Ees [96, 99-100]. A comparison of resting Ea/Ees in old (70 ± 8 yr, $n = 15$) and young (30 ± 5 yr, $n = 17$) subjects showed no significant differences due to comparable increase in both Ea and Ees (11 percent and 15 percent, respectively) in older subjects [101].

Hypertension

Hypertension is diagnosed when systolic blood pressure and diastolic blood pressure reach and exceed values of 140 mmHg and 80 mmHg respectively. If left untreated, over time hypertension can have deleterious effects on arterial and LV structure and function. For example, patients with hypertension have been shown to have greater carotid wall thickness, central arterial stiffness and greater wave reflection compared to normotensive patients [102]. In addition, increased wall tension and wall tensile stress elicit pathological changes in LV structure [103]. General agreement exists in the literature regarding the impact of hypertension on arterial-ventricular coupling with

hypertensive subjects displaying significant increases in Ea and Ees compared to normotensive subjects [104-105]. Notably, these same studies report no differences in the ratio of Ea/Ees indicating matched increases in Ea and Ees in hypertensive subjects.

Obesity

Deleterious changes that occur with obesity have been well documented within the literature including reduced arterial distensibility [106] and increased AS [106-107]. Further, obesity in the absence of CVD has been shown to cause changes in cardiac geometry including increased LV mass and LV volumes [108]. In terms of how changes in arterial and cardiac structure and function due to obesity affect arterial-ventricular coupling and its components, recent evidence has shown that when Ea and Ees are scaled ratiometrically or allometrically to body surface area, there is a significant increase in Ea and Ees in obese vs. non-obese controls [109]. The same study reported no change in the ratio of Ea/Ees between obese and non-obese controls, irrespective of body size [109].

Arterial-ventricular coupling and its components are essential determinants of the interactions between the heart and arteries. However, to date, very few studies in humans have examined the impact of MetS, free from T2DM and/or overt CVD, on cardiac energetics at rest or during exercise. This manuscript will set out to examine how the presence of MetS will impact arterial-ventricular coupling and its components at rest and during exercise.

Metabolic Syndrome and Exercise Training

Recommendations from the American Heart Association (AHA) for the management of MetS encourage therapeutic lifestyle changes to target the negative effects of atherogenic diet, weight gain, and sedentary lifestyle behavior. Exercise training has been reported to prevent and/or partly reverse MetS, however the most optimal exercise (i.e. type, duration, frequency, and intensity) for the treatment of MetS remains unresolved [110]. The following section will focus on the role of exercise training in the prevention and treatment of MetS and its individual components.

Obesity

Significant excess body weight ($\text{BMI} \geq 30 \text{ kg/m}^2$) may occur as a result of a long-term imbalance between energy intake and energy utilization. In the US the cholesterol-rich western diet and declining rates in physical activity in combination with long-term caloric excess have created an obesity epidemic. Evidence from scientific literature encourages the use of regular exercise to manage weight and decrease the long-term risk of obesity-related diseases including MetS and type II diabetes. Central adiposity or abdominal obesity, expressed as waist circumference (WC), is a key feature of MetS and is strongly associated with the risk of incident CVD events [111]. In a randomized, controlled study comparing diet-induced weight loss and exercise-induced weight loss, Ross et al. enrolled obese ($\text{BMI} \geq 27 \text{ kg/m}^2$ and $\text{WC} > 100 \text{ cm}$), sedentary men ($n=101$) and reported a greater significant reduction in total body fat following 12

weeks of exercise-induced weight loss [112]. Further, exercise-without-weight-loss showed a reduction in abdominal and visceral fat, which is closely related to WC and BMI [112]. Similarly, the authors of Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE-AT/RT) [113], a large randomized clinical trial of obese and overweight adults, found that WC was significantly reduced after combined aerobic/resistance exercise training vs. resistance training alone; Aerobic training alone produced a trend toward a significant decrease in WC. In a second publication from the STRRIDE study [114], all three eight-month aerobic exercise-training groups (high amount/vigorous intensity, low amount/vigorous intensity, and low amount/moderate intensity) displayed significant reductions in weight loss, fat loss, and waist and hip circumference measurements despite no significant changes in dietary intake for any group. These authors concluded that in non-dieting, mildly obese individuals a modest amount of moderate-intensity exercise (e.g. 30 minutes per day) is necessary to reverse a positive caloric imbalance and maintain body weight. In a small pilot study [115], 32 patients with MetS were recruited to address the efficacy of two modes of exercise to reverse features of MetS. Participants were randomized to equal volumes of either continuous moderate exercise or aerobic interval training for 16 weeks. Following 16 weeks of exercise training investigators reported exercise-induced reductions in WC and total body weight. Together with reported improvements in lipogenesis (the process by which simple sugars are converted to fatty acids) and increased circulating levels of adiponectin, these data suggest reductions in intraabdominal

visceral obesity and improvements in fat metabolism following 16 weeks of high intensity aerobic exercise training.

Hypertension

Long-term hypertension (HTN) (SBP ≥ 140 mmHg and DBP ≥ 90 mmHg) is recognized as a strong independent predictor of CVD [116]. It has been estimated that 40 percent of prehypertensive (SBP levels of 120 to 139 mmHg and/or DBP levels of 80 to 89 mm Hg) US adults will progress to HTN [117]. Studies based on the evaluation of physical activity using survey results support the idea that physical fitness may play a role in the attenuation of the progression to HTN [118-119]. Results from a recent study that followed 13,953 men (20-90 yrs.) free from hypertension, CVD and cancer, over a 36-year period support the notion that cardiorespiratory fitness can delay the age-associated increase of BP over an adult lifespan [120]. These results underscore the potential for delaying age-associated changes in BP by improving physical fitness levels with regular exercise and are in agreement with national guidelines for the primary and secondary prevention of HTN [116]. Specifically, the American College of Sports Medicine and the AHA recommends at least 150 minutes of moderate intensity, 75 minutes of high intensity exercise, or a combination of the two each week supplemented with at least 30 minutes of aerobic activity per day per week [121]. Importantly, the use of different exercise modalities as well as the parameters of frequency, duration, and intensity may have varying effects on BP. Historically regular endurance training has been the preferred physical activity for producing

favorable changes in BP [122-125]. Recent evidence from a meta-analysis of randomized controlled trials to compare the effects of various exercise modalities on the magnitude of changes in SBP and DBP in healthy adults found that endurance training, dynamic resistance training, and isometric resistance training significantly reduce BP and DBP [126]. Further, this analysis demonstrated that no significant differences in effect size were observed between endurance training and dynamic resistance training, however, the authors report that in hypertensive individuals, the greatest reductions in BP occurred after dynamic endurance training compared to other exercise modalities. A subgroup analysis of endurance training revealed that exercise-training programs of less than six months resulted in larger reductions in BP compared to programs of a longer duration. In terms of training intensity, low-intensity endurance training produced smaller BP reductions than those observed following moderate- or high-intensity endurance exercise [126].

Dyslipidemia

Dyslipidemia describes a disorder of lipoprotein metabolism. In MetS, dyslipidemia manifests as elevated total cholesterol, increased low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein (HDL-C), and elevations in plasma triglyceride (TG) concentrations. Studies focused on identifying the effects of exercise training and blood lipid profiles report dose-dependent relationships between exercise training volume and favorable changes in blood lipids [127]. Specifically, data from cross-sectional studies have

identified increases (2-3 mg/dL) in HDL-C and decreases in TG (8-20 mg/dL) following exercise training designed to elicit between 1200 to 2200 kcal/week [127]. Moderate intensity walking or jogging approximately 15-20 miles per week would be an appropriate stimulus to meet weekly caloric expenditures required to elicit favorable change in blood lipids [127]. Data from a meta-analysis of randomized controlled trials examining the effects of aerobic exercise on lipid and lipoproteins in overweight and obese adults is less convincing with trending, but statistically nonsignificant beneficial increases (3%) in HDL-C [128]. In contrast, evidence from the same meta-analysis suggests that aerobic exercise training produces a statistically significant (11%, $p < 0.05$) reduction in TG which remained significant even after removal of studies in which changes in diet, smoking status, and use of drugs could produce changes in the blood lipid profile [128]. In contrast to these reports, data from the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study [129], reveal a beneficial effect (+4.9%, $p < 0.005$) of 20 weeks of aerobic exercise training on men with high TG/low HDL-C and visceral adipose tissue. In men with isolated low HDL-C and normolipidemia 20 weeks of aerobic exercise training was unable to produce a significant increase in HDL-C (+0.4%, $P = \text{NS}$) [129]. While the lack of an effect in men with isolated HDL-C may seem discouraging, the “HDL-raising” effect observed in men with unfavorable blood lipids and abdominal obesity highlight the potential for exercise training to produce favorable changes in individuals with two or more cardiovascular risk factors as is seen in MetS.

Impaired Fasting Plasma Glucose

Under normal physiological conditions, the body responds to elevations in blood glucose concentrations by signaling the production and release of insulin by the pancreas. After binding to cell-surface receptors in skeletal muscle, fat, and liver, insulin initiates the uptake and storage of glucose, thereby playing a key role in the regulation of glucose homeostasis. In MetS, insulin resistance can result in chronically elevated blood sugar and if left untreated, the development of T2DM. Exercise is often employed as a lifestyle-related intervention to reverse and/or control elevations in blood glucose through improvements in insulin sensitivity. Data from human studies suggest significant improvements in fasting plasma glucose and insulin sensitivity in individuals with impaired glucose tolerance following aerobic exercise training. A recent investigation examined adaptations in insulin sensitivity (HOMA) in response to continuous moderate exercise or high intensity aerobic interval training in individuals with MetS [115]. When compared to continuous moderate exercise, high intensity aerobic exercise training 3x/week for 16 weeks was shown to be superior at improving insulin sensitivity (HOMA) in MetS [115]. The observed benefits of high intensity exercise training above and beyond moderate intensity exercise training highlights the importance of exercise intensity for targeting improvements in insulin action and glucose homeostasis. Several human studies have demonstrated similar findings and suggest an important role for substrate utilization. For example, high intensity exercise contributes to the use of intramuscular glycogen as a reserve source of available glucose for fuel. The

post-exercise actions of insulin include stimulation of glucose uptake by the liver and muscle cells and subsequent glycogen synthesis to replenish depleted intramuscular glycogen. Evidence from animals studies using a rat model of MetS demonstrates that treadmill training for 1 hour/day, 5 days/week for 10 weeks reduced blood pressure, improved insulin sensitivity, and increased glucose transporter Type 4 (GLUT4) content in the heart, white adipose tissue, and gastrocnemius muscle relative to untrained animals [130]. Exercise training-induced improvements in insulin sensitivity are believed to be mediated from increases in GLUT4 gene expression [131] as well as from improved translocation of GLUT4 from the cytoplasm to the cell membrane [132-133].

Metabolic Syndrome

When considering the efficacy of a therapeutic lifestyle change for the management of MetS, it is important to take into account not only the effects of exercise training on each individual risk factor but to focus on reversing multiple risk factors for the prevention and improvement of MetS. Evidence from the STRRIDE study examining the effects of different volumes of exercise training on multiple components of MetS reveal that both shorter duration/moderate intensity and longer duration/vigorous intensity had significantly reductions in ATP III and MS z scores compared to inactive controls [134]. Compared to moderate-intensity exercise, vigorous-intensity exercise produced a greater number of improved components of MetS [134] however, a modest amount of moderate-intensity exercise was adequate to obtain metabolic health benefits.

Comparatively, a second publication from the STRRIDE study addressed the comparison of cardiometabolic benefits of aerobic training, resistance training, and combined training in MetS [113]. Investigators reported significant improvements in the MS z score following combined exercise training and a trend toward improvement following aerobic training, while resistance training failed to have any effect on MS z score [113]. Despite the reported benefits of combined exercise training in MetS, the authors concluded that aerobic exercise training represented the most efficient mode of exercise training to address the cardiometabolic risk factors associated with MetS citing that the argument for combined exercise training was weak when considering the time commitment vs. cardiometabolic health benefits [113]. Similarly, findings from the HERITAGE Family Study to determine the efficacy of aerobic exercise training for treating MetS, provide evidence of the efficacy of physical activity in treating individuals with multiple cardiovascular risk factors [135]. The HERITAGE STUDY recruited 288 men and 333 women who were evaluated for MetS to participate in a 20-week ramp-style aerobic exercise-training program. Interestingly the overall prevalence of MetS decreased from 16.9 percent to 11.8 percent following exercise training. Of the total number of participants with MetS at the start of the study, 30.5 percent were no longer classified as meeting the NCEP diagnostic criteria for MetS following training. Even more encouraging is the fact that this study did not include individuals with overt cardiovascular disease or hypertension and thus, the observed benefits of aerobic exercise in the treatment of MetS may play an important role in the prevention of the progression of

subclinical metabolic disease to overt chronic disease [135]. The results of the HERITAGE Family Study and others strongly support the NCEP ATP III recommendations for the use of therapeutic lifestyle modifications (aerobic exercise training) for the treatment of individuals with MetS.

While the benefits of exercise training on reversing and preventing the progression of MetS to overt CVD are well agreed upon, the mechanisms underlying exercise training-induced adaptations in MetS are unclear. Aerobic exercise training has been established as an effective intervention for improving arterial stiffness, increasing aerobic capacity, and improving peak LV performance and LV contractility in healthy persons as well as in older persons with overt CVD, including T2DM and coronary artery disease. However it is unknown whether aerobic exercise training can improve arterial stiffening and peak LV-arterial coupling and CV function in MetS, which would likely reflect a reduction in CVD risk. In this document we will demonstrate the benefits of aerobic exercise training on arterial stiffness and arterial-ventricular coupling and its components in MetS free from T2DM and/or overt CVD.

Reference List

1. Beltrán-Sánchez, H., et al., *Prevalence and Trends of Metabolic Syndrome in the Adult U.S. Population, 1999–2010*. Journal of the American College of Cardiology, 2013. **62**(8): p. 697-703.
2. James, P.T., N. Rigby, and R. Leach, *The obesity epidemic, metabolic syndrome and future prevention strategies*. Eur J Cardiovasc Prev Rehabil, 2004. **11**(1): p. 3-8.
3. Go, A.S., et al., *Heart Disease and Stroke Statistics—2014 Update: A Report From the American Heart Association*. Circulation, 2014. **129**(3): p. e28-e292.
4. McNeill, A.M., et al., *Metabolic syndrome and cardiovascular disease in older people: The cardiovascular health study*. J Am Geriatr Soc, 2006. **54**(9): p. 1317-24.
5. Wilson, P.W., et al., *Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus*. Circulation, 2005. **112**(20): p. 3066-72.
6. Vyssoulis, G.P., et al., *Differential impact of metabolic syndrome on arterial stiffness and wave reflections: focus on distinct definitions*. Int J Cardiol, 2010. **138**(2): p. 119-25.
7. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. **43**(8): p. 1388-95.

8. Lewington, S., et al., *Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies*. Lancet, 2002. **360**(9349): p. 1903-13.
9. Lakatta, E.G., *Cardiovascular regulatory mechanisms in advanced age*. Physiol Rev, 1993. **73**(2): p. 413-67.
10. Langille, B.L., *Arterial remodeling: relation to hemodynamics*. Can J Physiol Pharmacol, 1996. **74**(7): p. 834-41.
11. O'Leary, D.H., et al., *Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults*. Cardiovascular Health Study Collaborative Research Group. N Engl J Med, 1999. **340**(1): p. 14-22.
12. Nagai, Y., et al., *Increased carotid artery intimal-medial thickness in asymptomatic older subjects with exercise-induced myocardial ischemia*. Circulation, 1998. **98**(15): p. 1504-9.
13. Li, Z., et al., *Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats*. Hypertension, 1999. **33**(1): p. 116-23.
14. Asai, K., et al., *Peripheral vascular endothelial dysfunction and apoptosis in old monkeys*. Arterioscler Thromb Vasc Biol, 2000. **20**(6): p. 1493-9.
15. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. Exp Physiol, 2013.
16. Ferreira, I., et al., *Clustering of metabolic syndrome traits is associated with maladaptive carotid remodeling and stiffening: a 6-year longitudinal study*. Hypertension, 2012. **60**(2): p. 542-9.

17. Conen, D., et al., *Metabolic syndrome, inflammation, and risk of symptomatic peripheral artery disease in women: a prospective study*. Circulation, 2009. **120**(12): p. 1041-7.
18. Lakatta, E.G. and D. Levy, *Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease*. Circulation, 2003. **107**(1): p. 139-46.
19. Vaitkevicius, P.V., et al., *Effects of age and aerobic capacity on arterial stiffness in healthy adults*. Circulation, 1993. **88**(4 Pt 1): p. 1456-62.
20. Safar, M.E., et al., *Metabolic syndrome and age-related progression of aortic stiffness*. J Am Coll Cardiol, 2006. **47**(1): p. 72-5.
21. Najjar, S.S., A. Scuteri, and E.G. Lakatta, *Arterial aging: is it an immutable cardiovascular risk factor?* Hypertension, 2005. **46**(3): p. 454-62.
22. Azevedo, A., et al., *Increasing number of components of the metabolic syndrome and cardiac structural and functional abnormalities--cross-sectional study of the general population*. BMC Cardiovasc Disord, 2007. **7**: p. 17.
23. Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis, *Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis*. J Am Coll Cardiol, 2010. **55**(13): p. 1318-27.
24. Dao, H.H., et al., *Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension*. Cardiovasc Res, 2005. **66**(2): p. 307-17.

25. Zieman, S.J., V. Melenovsky, and D.A. Kass, *Mechanisms, pathophysiology, and therapy of arterial stiffness*. Arterioscler Thromb Vasc Biol, 2005. **25**(5): p. 932-43.
26. Boluyt, M.O. and O.H. Bing, *Matrix gene expression and decompensated heart failure: the aged SHR model*. Cardiovasc Res, 2000. **46**(2): p. 239-49.
27. Galis, Z.S. and J.J. Khatri, *Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly*. Circ Res, 2002. **90**(3): p. 251-62.
28. Hingorani, A.D., et al., *Acute systemic inflammation impairs endothelium-dependent dilatation in humans*. Circulation, 2000. **102**(9): p. 994-999.
29. Spinale, F.G., et al., *Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: relation to ventricular and myocyte function*. Circ Res, 1998. **82**(4): p. 482-95.
30. Zhang, J., et al., *Molecular imaging of activated matrix metalloproteinases in vascular remodeling*. Circulation, 2008. **118**(19): p. 1953-60.
31. Rodriguez- Pla, A., et al., *Metalloproteinase-2 and-9 in giant cell arteritis - Involvement in vascular remodeling*. Circulation, 2005. **112**(2): p. 264-269.
32. Bouvet, C., et al., *Sequential activation of matrix metalloproteinase 9 and transforming growth factor beta in arterial elastocalcinosis*. Arterioscler Thromb Vasc Biol, 2008. **28**(5): p. 856-62.

33. Ammarguellat, F.Z., et al., *Fibrosis, matrix metalloproteinases, and inflammation in the heart of DOCA-salt hypertensive rats: role of ET(A) receptors*. Hypertension, 2002. **39**(2 Pt 2): p. 679-84.
34. Laviades, C., et al., *Abnormalities of the extracellular degradation of collagen type I in essential hypertension*. Circulation, 1998. **98**(6): p. 535-40.
35. Tan, J., et al., *Impact of the metalloproteinase-9/tissue inhibitor of metalloproteinase-1 system on large arterial stiffness in patients with essential hypertension*. Hypertens Res, 2007. **30**(10): p. 959-63.
36. Tayebjee, M.H., et al., *Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)*. Am J Hypertens, 2004. **17**(9): p. 764-9.
37. Wilkinson, I.B. and C.M. McEniery, *Arterial stiffness, endothelial function and novel pharmacological approaches*. Clin Exp Pharmacol Physiol, 2004. **31**(11): p. 795-9.
38. Boutouyrie, P., et al., *Sympathetic activation decreases medium-sized arterial compliance in humans*. Am J Physiol, 1994. **267**(4 Pt 2): p. H1368-76.
39. Joannides, R., et al., *Influence of sympathetic tone on mechanical properties of muscular arteries in humans*. Am J Physiol, 1995. **268**(2 Pt 2): p. H794-801.

40. Lydakis, C., et al., *Changes of elastic properties of central arteries during acute static exercise and lower body negative pressure*. Eur J Appl Physiol, 2008. **102**(6): p. 633-41.
41. Kinlay, S., et al., *Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo*. Hypertension, 2001. **38**(5): p. 1049-53.
42. McEniery, C.M., et al., *Endothelin-1 regulates arterial pulse wave velocity in vivo*. J Am Coll Cardiol, 2003. **42**(11): p. 1975-81.
43. Wilkinson, I.B., S.S. Franklin, and J.R. Cockcroft, *Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology*. Hypertension, 2004. **44**(2): p. 112-6.
44. Mudau, M., et al., *Endothelial dysfunction: the early predictor of atherosclerosis*. Cardiovasc J Afr, 2012. **23**(4): p. 222-31.
45. Suwaidi, J.A., et al., *Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction*. Circulation, 2000. **101**(9): p. 948-54.
46. Palmer, R.M., D.S. Ashton, and S. Moncada, *Vascular endothelial cells synthesize nitric oxide from L-arginine*. Nature, 1988. **333**(6174): p. 664-6.
47. Anderson, T.J., *Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk*. Can J Cardiol, 2006. **22 Suppl B**: p. 72B-80B.
48. Le Brocq, M., et al., *Endothelial dysfunction: from molecular mechanisms to measurement, clinical implications, and therapeutic opportunities*. Antioxid Redox Signal, 2008. **10**(9): p. 1631-74.

49. Flammer, A.J., et al., *The assessment of endothelial function: from research into clinical practice*. Circulation, 2012. **126**(6): p. 753-67.
50. Esposito, K., et al., *Effect of rosiglitazone on endothelial function and inflammatory markers in patients with the metabolic syndrome*. Diabetes Care, 2006. **29**(5): p. 1071-6.
51. Hamburg, N.M., et al., *Metabolic syndrome, insulin resistance, and brachial artery vasodilator function in Framingham Offspring participants without clinical evidence of cardiovascular disease*. Am J Cardiol, 2008. **101**(1): p. 82-8.
52. Benjamin, E.J., et al., *Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study*. Circulation, 2004. **109**(5): p. 613-9.
53. Brook, R.D., et al., *Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults*. Am J Cardiol, 2001. **88**(11): p. 1264-9.
54. Kuvin, J.T., et al., *Relation between high-density lipoprotein cholesterol and peripheral vasomotor function*. Am J Cardiol, 2003. **92**(3): p. 275-9.
55. Lupattelli, G., et al., *Flow-mediated vasoactivity and circulating adhesion molecules in hypertriglyceridemia: association with small, dense LDL cholesterol particles*. Am Heart J, 2000. **140**(3): p. 521-6.
56. Ford, E.S., *The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey*. Atherosclerosis, 2003. **168**(2): p. 351-8.

57. Demircan, N., et al., *The evaluation of serum cystatin C, malondialdehyde, and total antioxidant status in patients with metabolic syndrome*. Med Sci Monit, 2008. **14**(2): p. CR97-101.
58. Bakker, E.N., et al., *Flow-dependent remodeling of small arteries in mice deficient for tissue-type transglutaminase: possible compensation by macrophage-derived factor XIII*. Circ Res, 2006. **99**(1): p. 86-92.
59. Malik, S., et al., *Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults*. Circulation, 2004. **110**(10): p. 1245-1250.
60. Voulgari, C., et al., *The impact of metabolic syndrome on left ventricular myocardial performance*. Diabetes Metab Res Rev, 2010. **26**(2): p. 121-7.
61. Chinali, M., et al., *Cardiac markers of pre-clinical disease in adolescents with the metabolic syndrome: the strong heart study*. J Am Coll Cardiol, 2008. **52**(11): p. 932-8.
62. Aijaz, B., et al., *Abnormal Cardiac Structure and Function in the Metabolic Syndrome: A Population-Based Study*. Mayo Clinic Proceedings, 2008. **83**(12): p. 1350-1357.
63. Azevedo, A., et al., *Increasing number of components of the metabolic syndrome and cardiac structural and functional abnormalities - cross-sectional study of the general population*. BMC Cardiovascular Disorders, 2007. **7**(1): p. 17.
64. Mahmud, A., et al., *Left ventricular structural and functional changes in the metabolic syndrome*. J Cardiometab Syndr, 2009. **4**(2): p. 81-8.

65. Page, A., et al., *Metabolic syndrome is associated with more pronounced impairment of left ventricle geometry and function in patients with calcific aortic stenosis: a substudy of the ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin)*. J Am Coll Cardiol, 2010. **55**(17): p. 1867-74.
66. Chinali, M., et al., *Comparison of cardiac structure and function in American Indians with and without the metabolic syndrome (the Strong Heart Study)*. Am J Cardiol, 2004. **93**(1): p. 40-4.
67. de las Fuentes, L., et al., *Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass*. Eur Heart J, 2007. **28**(5): p. 553-9.
68. Mule, G., et al., *Impact of metabolic syndrome on left ventricular mass in overweight and obese hypertensive subjects*. Int J Cardiol, 2007. **121**(3): p. 267-75.
69. Mule, G., et al., *Influence of metabolic syndrome on hypertension-related target organ damage*. J Intern Med, 2005. **257**(6): p. 503-13.
70. Aijaz, B., et al., *Abnormal cardiac structure and function in the metabolic syndrome: a population-based study*. Mayo Clin Proc, 2008. **83**(12): p. 1350-7.
71. Carabello, B.A., *Understanding coronary blood flow: the wave of the future*. Circulation, 2006. **113**(14): p. 1721-2.

72. Koren, M.J., et al., *Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension*. Ann Intern Med, 1991. **114**(5): p. 345-52.
73. Levy, D., et al., *Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study*. N Engl J Med, 1990. **322**(22): p. 1561-6.
74. von Bibra, H. and M. St John Sutton, *Diastolic dysfunction in diabetes and the metabolic syndrome: promising potential for diagnosis and prognosis*. Diabetologia, 2010. **53**(6): p. 1033-45.
75. Turhan, H., et al., *Impaired coronary blood flow in patients with metabolic syndrome: documented by Thrombolysis in Myocardial Infarction (TIMI) frame count method*. Am Heart J, 2004. **148**(5): p. 789-94.
76. Nyman, K., et al., *Cardiac steatosis and left ventricular function in men with metabolic syndrome*. J Cardiovasc Magn Reson, 2013. **15**: p. 103.
77. Pagé, A., et al., *Metabolic Syndrome Is Associated With More Pronounced Impairment of Left Ventricle Geometry and Function in Patients With Calcific Aortic Stenosis: A Substudy of the ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin)*. Journal of the American College of Cardiology, 2010. **55**(17): p. 1867-1874.
78. Wong, C.Y., et al., *Myocardial and vascular dysfunction and exercise capacity in the metabolic syndrome*. Am J Cardiol, 2005. **96**(12): p. 1686-91.

79. Gong, H.P., et al., *Impaired left ventricular systolic and diastolic function in patients with metabolic syndrome as assessed by strain and strain rate imaging*. Diabetes Res Clin Pract, 2009. **83**(3): p. 300-7.
80. Gong, H.-p., et al., *Impaired left ventricular systolic and diastolic function in patients with metabolic syndrome as assessed by strain and strain rate imaging*. Diabetes Research and Clinical Practice, 2009. **83**(3): p. 300-307.
81. De Tombe, P.P., et al., *Ventricular stroke work and efficiency both remain nearly optimal despite altered vascular loading*. Am J Physiol, 1993. **264**(6 Pt 2): p. H1817-24.
82. Najjar, S.S., et al., *Age and gender affect ventricular-vascular coupling during aerobic exercise*. J Am Coll Cardiol, 2004. **44**(3): p. 611-7.
83. Sunagawa, K., et al., *Left ventricular interaction with arterial load studied in isolated canine ventricle*. Am J Physiol, 1983. **245**(5 Pt 1): p. H773-80.
84. Sunagawa, K., W.L. Maughan, and K. Sagawa, *Optimal arterial resistance for the maximal stroke work studied in isolated canine left ventricle*. Circ Res, 1985. **56**(4): p. 586-95.
85. Borlaug, B.A. and D.A. Kass, *Ventricular-vascular interaction in heart failure*. Heart Fail Clin, 2008. **4**(1): p. 23-36.
86. Chantler, P.D. and E.G. Lakatta, *Arterial-ventricular coupling with aging and disease*. Front Physiol, 2012. **3**: p. 90.

87. Suga, H., *Theoretical analysis of a left-ventricular pumping model based on the systolic time-varying pressure-volume ratio*. IEEE Trans Biomed Eng, 1971. **18**(1): p. 47-55.
88. Suga, H., *Total mechanical energy of a ventricle model and cardiac oxygen consumption*. Am J Physiol, 1979. **236**(3): p. H498-505.
89. Suga, H., K. Sagawa, and A.A. Shoukas, *Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio*. Circ Res, 1973. **32**(3): p. 314-22.
90. Suga, H. and K. Sagawa, *Mathematical interrelationship between instantaneous ventricular pressure-volume ratio and myocardial force-velocity relation*. Ann Biomed Eng, 1972. **1**(2): p. 160-81.
91. Kass, D.A., *Age-related changes in ventricular-arterial coupling: pathophysiologic implications*. Heart Fail Rev, 2002. **7**(1): p. 51-62.
92. Chantler, P.D., E.G. Lakatta, and S.S. Najjar, *Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise*. J Appl Physiol, 2008. **105**(4): p. 1342-51.
93. Segers, P., N. Stergiopulos, and N. Westerhof, *Relation of effective arterial elastance to arterial system properties*. Am J Physiol Heart Circ Physiol, 2002. **282**(3): p. H1041-6.
94. Kelly, R.P., et al., *Effective arterial elastance as index of arterial vascular load in humans*. Circulation, 1992. **86**(2): p. 513-21.

95. Burkhoff, D. and K. Sagawa, *Ventricular efficiency predicted by an analytical model*. Am J Physiol, 1986. **250**(6 Pt 2): p. R1021-7.
96. Chen, C.H., et al., *Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly*. J Am Coll Cardiol, 1998. **32**(5): p. 1221-7.
97. Little, W.C. and C.P. Cheng, *Effect of exercise on left ventricular-arterial coupling assessed in the pressure-volume plane*. Am J Physiol, 1993. **264**(5 Pt 2): p. H1629-33.
98. Otsuki, T., et al., *Contribution of systemic arterial compliance and systemic vascular resistance to effective arterial elastance changes during exercise in humans*. Acta Physiol (Oxf), 2006. **188**(1): p. 15-20.
99. Cohen-Solal, A., et al., *Effects of aging on left ventricular-arterial coupling in man: assessment by means of arterial effective and left ventricular elastances*. J Hum Hypertens, 1996. **10**(2): p. 111-6.
100. Redfield, M.M., et al., *Age- and gender-related ventricular-vascular stiffening: a community-based study*. Circulation, 2005. **112**(15): p. 2254-62.
101. Chantler, P.D., et al., *Abnormalities in arterial-ventricular coupling in older healthy persons are attenuated by sodium nitroprusside*. Am J Physiol Heart Circ Physiol, 2011. **300**(5): p. H1914-22.
102. Nichols, W.W., F.A. Nicolini, and C.J. Pepine, *Determinants of isolated systolic hypertension in the elderly*. J Hypertens Suppl, 1992. **10**(6): p. S73-7.

103. Mayet, J. and A. Hughes, *Cardiac and vascular pathophysiology in hypertension*. Heart, 2003. **89**(9): p. 1104-9.
104. Cohen-Solal, A., et al., *Left ventricular-arterial coupling in systemic hypertension: analysis by means of arterial effective and left ventricular elastances*. J Hypertens, 1994. **12**(5): p. 591-600.
105. Saba, P.S., et al., *Impact of arterial elastance as a measure of vascular load on left ventricular geometry in hypertension*. J Hypertens, 1999. **17**(7): p. 1007-15.
106. Zebekakis, P.E., et al., *Obesity is associated with increased arterial stiffness from adolescence until old age*. J Hypertens, 2005. **23**(10): p. 1839-46.
107. Toto-Moukouro, J.J., et al., *Pulse wave velocity in patients with obesity and hypertension*. Am Heart J, 1986. **112**(1): p. 136-40.
108. Rider, O.J., et al., *Ventricular hypertrophy and cavity dilatation in relation to body mass index in women with uncomplicated obesity*. Heart, 2011. **97**(3): p. 203-8.
109. Chirinos, J.A., et al., *Arterial load and ventricular-arterial coupling: physiologic relations with body size and effect of obesity*. Hypertension, 2009. **54**(3): p. 558-66.
110. Lakka, H.M., et al., *The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men*. JAMA, 2002. **288**(21): p. 2709-16.

111. de Koning, L., et al., *Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies*. Eur Heart J, 2007. **28**(7): p. 850-6.
112. Ross, R., et al., *Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial*. Ann Intern Med, 2000. **133**(2): p. 92-103.
113. Bateman, L.A., et al., *Comparison of aerobic versus resistance exercise training effects on metabolic syndrome (from the Studies of a Targeted Risk Reduction Intervention Through Defined Exercise - STRRIDE-AT/RT)*. Am J Cardiol, 2011. **108**(6): p. 838-44.
114. Slentz, C.A., et al., *Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE--a randomized controlled study*. Arch Intern Med, 2004. **164**(1): p. 31-9.
115. Tjonna, A.E., et al., *Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study*. Circulation, 2008. **118**(4): p. 346-54.
116. Chobanian, A.V., et al., *The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report*. JAMA, 2003. **289**(19): p. 2560-72.
117. Mann, J., *[Treatment of high-normal blood pressure with angiotensin receptor blockers. TROPHY Study (Trial of Preventing Hypertension)]*. Internist (Berl), 2007. **48**(4): p. 436-8.

118. Blair, S.N., et al., *Physical fitness and incidence of hypertension in healthy normotensive men and women*. JAMA, 1984. **252**(4): p. 487-90.
119. Parker, E.D., et al., *Physical activity in young adults and incident hypertension over 15 years of follow-up: the CARDIA study*. Am J Public Health, 2007. **97**(4): p. 703-9.
120. Liu, J., et al., *Effects of cardiorespiratory fitness on blood pressure trajectory with aging in a cohort of healthy men*. J Am Coll Cardiol, 2014. **64**(12): p. 1245-53.
121. Haskell, W.L., et al., *Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association*. Med Sci Sports Exerc, 2007. **39**(8): p. 1423-34.
122. Cornelissen, V.A. and R.H. Fagard, *Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors*. Hypertension, 2005. **46**(4): p. 667-75.
123. Collier, S.R., et al., *Effect of 4 weeks of aerobic or resistance exercise training on arterial stiffness, blood flow and blood pressure in pre- and stage-1 hypertensives*. J Hum Hypertens, 2008. **22**(10): p. 678-86.
124. Whelton, S.P., et al., *Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials*. Ann Intern Med, 2002. **136**(7): p. 493-503.
125. Higashi, Y., et al., *Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive*

- subjects: role of endothelium-derived nitric oxide.* Circulation, 1999. **100**(11): p. 1194-202.
126. Cornelissen, V.A. and N.A. Smart, *Exercise training for blood pressure: a systematic review and meta-analysis.* J Am Heart Assoc, 2013. **2**(1): p. e004473.
 127. Durstine, J.L., et al., *Blood lipid and lipoprotein adaptations to exercise: a quantitative analysis.* Sports Med, 2001. **31**(15): p. 1033-62.
 128. Kelley, G.A., K.S. Kelley, and Z. Vu Tran, *Aerobic exercise, lipids and lipoproteins in overweight and obese adults: a meta-analysis of randomized controlled trials.* Int J Obes (Lond), 2005. **29**(8): p. 881-93.
 129. Couillard, C., et al., *Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study.* Arterioscler Thromb Vasc Biol, 2001. **21**(7): p. 1226-32.
 130. Caponi, P.W., et al., *Aerobic exercise training induces metabolic benefits in rats with metabolic syndrome independent of dietary changes.* Clinics (Sao Paulo), 2013. **68**(7): p. 1010-7.
 131. Friedman, J.E., et al., *Exercise training increases glucose transporter protein GLUT-4 in skeletal muscle of obese Zucker (fa/fa) rats.* FEBS Lett, 1990. **268**(1): p. 13-6.
 132. Ren, J.M., et al., *Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle.* J Biol Chem, 1994. **269**(20): p. 14396-401.

133. Neufer, P.D., M.H. Shinebarger, and G.L. Dohm, *Effect of training and detraining on skeletal muscle glucose transporter (GLUT4) content in rats.* Can J Physiol Pharmacol, 1992. **70**(9): p. 1286-90.
134. Johnson, J.L., et al., *Exercise training amount and intensity effects on metabolic syndrome (from Studies of a Targeted Risk Reduction Intervention through Defined Exercise).* Am J Cardiol, 2007. **100**(12): p. 1759-66.
135. Katzmarzyk, P.T., et al., *Targeting the metabolic syndrome with exercise: evidence from the HERITAGE Family Study.* Med Sci Sports Exerc, 2003. **35**(10): p. 1703-9.
136. Chantler, P.D., E.G. Lakatta, and S.S. Najjar, *Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise.* Journal of Applied Physiology, 2008. **105**(4): p. 1342-1351.

Figure Legends

Figure 1. Synthesis, activation, and signaling of NO for smooth muscle cell relaxation.

Figure 2. This figure was modified from Chantler et al. [136]. Pressure-volume relationships illustrating the slope of the end-systolic pressure volume relationship (E_{es}), and the slope of the line joining end-systolic pressure and end-diastolic volume points (E_a) in healthy controls.

Figure 1.

Figure 2.

Chapter 2

EXERCISE REVEALS IMPAIRMENTS IN LEFT VENTRICULAR SYSTOLIC FUNCTION IN PATIENTS WITH METABOLIC SYNDROME

Sara B. Fournier^{1,2}, Brian L. Reger¹, David A. Donley¹, Daniel E. Bonner¹,
Bradford E. Warden^{2,3}, Wissam Gharib^{2,3}, Conrad F. Failingner², Melissa D. Olfert⁴,
Jefferson C. Frisbee^{2,5}, I. Mark Olfert^{1,2}, and Paul D. Chantler^{1,2}

¹Division of Exercise Physiology, School of Medicine, West Virginia University;

²Center for Cardiovascular and Respiratory Sciences, School of Medicine, West

Virginia University; ³Division of Cardiology, School of Medicine, West Virginia

University. ⁴Human Nutrition and Foods, Davis College of Agriculture, West

Virginia University. ⁵Department of Physiology and Pharmacology, School of

Medicine, West Virginia University.

Running Head: Exercise Reveals LV Systolic Dysfunction in MetS

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Abstract

Metabolic syndrome (MetS) is the manifestation of a cluster of cardiovascular (CV) risk factors and is associated with a three-fold increase risk of CV morbidity and mortality, which is suggested to be mediated, in part, by resting left ventricular (LV) systolic dysfunction. However, to what extent resting LV systolic function is impaired in MetS is controversial, and there are no data indicating whether LV systolic function is impaired during exercise. Accordingly, the objective of this study was to comprehensively examine LV and arterial responses to exercise in MetS individuals without diabetes and/or overt CVD compared to a healthy control population. CV function was characterized using Doppler echocardiography and gas exchange in MetS (n=27) vs. healthy controls (n=20) at rest and during peak exercise. At rest, MetS individuals displayed normal LV systolic function but reduced LV diastolic function vs. healthy controls. During peak exercise, individuals with MetS had impaired contractility; pump performance, and vasodilator reserve capacity vs. controls. A blunted contractile reserve response resulted in diminished arterial-ventricular coupling reserve and limited aerobic capacity in MetS vs. controls. These findings possess clinical importance as they provide insight to the pathophysiological changes in MetS that may predispose this population of individuals to an increased risk of CV morbidity and mortality.

Introduction

Individuals with the metabolic syndrome (MetS) are at a three-fold increased risk of cardiovascular disease (CVD) mortality than non-MetS individuals [1]. Alarming, the prevalence of MetS in US adults is 34 percent and is on the rise due, in part, to rising rates of obesity [2], which will likely lead to a further increase in CVD incidence. This increased CVD mortality in MetS may be mediated, in part, by impaired LV systolic function that has been noted at rest in some studies [3-6] but not all [7, 8]. However, many of the studies used load-dependent measures of systolic function (endocardial or midwall fractional shortening) or ejection fraction, which is more representative of the interaction between the LV and arterial system [9], as measures of LV systolic function (Azevedo *et al.*, 2007; Aijaz *et al.*, 2008; Mahmud *et al.*, 2009; de las Fuentes *et al.*, 2007). Further, the populations examined in these studies were confounded by including MetS patients with diabetes and/or overt CVD [7, 10, 11], and/or comparing MetS individuals to a control group that contained individuals with CVD [3-7, 11]. Such confounding factors do not allow interpretations as to whether the severity of the changes in cardiovascular (CV) function is representative of MetS or other pathologies.

Importantly, existing evidence of LV dysfunction in MetS is limited to resting situations, where the CV system is attempting to optimize chamber pumping efficiency [12]. In contrast, during exercise the CV system is set to prioritize the output of the heart over energetic efficiency by coordinating changes in LV function, arterial tone, endothelial function, venous return, and

autonomic signaling. Thus, it could be expected that LV abnormalities, undetectable at rest, may appear with exertion to limit exercise capacity in MetS and contribute to an impaired quality of life [13]. However, to date, the pathophysiological alterations in LV function, and the degree to which these changes influence the interaction of the heart with the arterial system during exercise in MetS is unknown. Thus, examining the LV functional changes during exercise may further provide pathophysiological insights into the CVD risk in MetS patients. Accordingly, the aim of this study was to determine the severity of LV and arterial dysfunction during exercise in MetS individuals without diabetes and/or overt CVD compared to a healthy control population. Further, we will also examine resting LV systolic and diastolic function between MetS and healthy controls. We hypothesize that MetS individuals will have an impaired LV contractile response (peak end-systolic elastance and pre-load recruitable stroke work) to maximal exercise, leading to a blunted arterial-ventricular coupling response during exercise compared to healthy age-sex matched controls.

Methods

Study Population

Twenty-seven subjects with MetS were recruited into the study. MetS was defined according to the updated National Cholesterol Education Program: Adult Treatment Panel III [14] comprised of three out of the following five components: 1) obesity (waist men >102 cm, women >88 cm); 2) low HDL cholesterol (men <40 mg/dL; women <50 mg/dL); 3) hypertriglyceridemia (≥ 150 mg/dL); 4) elevated glucose (≥ 100 mg/dL), and; 5) elevated BP (130/85 mmHg or use of hypertensive medications). The MetS population was compared to twenty healthy controls free from CVD, as determined by a detailed history, physical examination, and a normal resting and exercise electrocardiogram. Exclusion criteria for MetS and healthy controls included abnormal resting and/or exercise electrocardiogram, diabetes mellitus (HbA1c ≥ 6.5 % or use of diabetic medications), pulmonary disease, angina, atrial fibrillation, aortic stenosis (or other types of valve disease), anemia, myocardial infarction, stroke, or coronary revascularization as assessed by a detailed medical history, and physical examination. No evidence of wall motion abnormalities was identified during the maximal exercise test. Subjects who participated in regular exercise, defined as greater than 30 minutes, three times/week were excluded to ensure similar physical activity levels between groups. All subjects provided written informed consent to participate that was approved by WVU Institutional Review Board.

Study Design

Physiological assessments were performed between 7:00-10:00 AM, in a quiet, temperature-controlled room, after a 12-hour fast and abstinence from alcohol, caffeine, and vitamins. CV medications were withheld 24 hours prior to assessments. After a minimum 15 minutes of quiet rest subjects underwent supine, resting, non-invasive assessments of arterial and cardiac structure and function. Once supine assessments were completed, subjects moved to a modified monarch bike (Figure 1) where upright rest and exercise measures of CV function were obtained.

Exercise Performance

Subjects underwent upright cycle maximal exercise testing. To optimize acquisition of the echo images, the back support of the cycle was set at approximately 130 degrees. Pedal speed was maintained at 50 rpm, and workloads increased by 25 W every 3 minutes until exhaustion. Oxygen consumed (VO_2), carbon dioxide produced (VCO_2), and the respiratory exchange ratio ($\text{RER}=\text{VCO}_2/\text{VO}_2$) were measured (ParvoMedics) throughout exercise. Subjective symptoms of fatigue (BORG score 6 to 20) [15], and BP's (sphygmomanometry) were recorded at the end of each workload. Registered diagnostic cardiac sonographers acquired transthoracic echo-Doppler/tissue Doppler images during the final 1.5 minutes of each 3-minute exercise workload (GE Vivid i, GE Healthcare, Chalfont St. Giles, United Kingdom).

Cardiovascular Measurements

Arterial Geometry and stiffness: In the supine position, ultrasound (GE Vivid i) 2-D images of the right common carotid artery were obtained 1-2 cm proximal to the carotid bifurcation to measure maximal lumen diameter, and intima-medial thickness (cIMT) following standard procedures [16]. Cross-sectional area of the carotid artery was calculated as $[(\text{maximal lumen diameter}/2)^2 \times \pi] - (\text{maximal lumen diameter}/2 - \text{cIMT})^2 \times \pi$. Carotid circumferential stress was calculated as systolic BP $\times$ (carotid diameter in diastole/2). Carotid to femoral pulse wave velocity (cfPWV; central arterial stiffness) was measured by applanation tonometry (AtCor Medical, Sydney, Australia) [17]. ECG-gated waveforms were sequentially recorded. Aortic distance (D) was calculated as the difference in the distances from the carotid to the suprasternal notch and from the suprasternal notch to the femoral artery. Time delay was calculated using a foot-of-the-wave method.

Left Ventricular Geometry and Remodeling: In the supine position, LV dimensions, wall thickness, and chamber volumes were determined in triplicate from 2-D, M-mode, and Doppler spectra echocardiography using standard methods [18]. Sex-specific LV hypertrophy (LVH) and geometry patterns based on LV mass index and relative wall thickness (RWT) was defined as LV mass index $>95 \text{ g/m}^2$ for women or $>115 \text{ g/m}^2$ for men, and LV geometry was classified as normal, concentric remodeling, concentric LVH, or eccentric LVH [18].

Resting Diastolic Function: In the supine position, the medial mitral annular early diastolic velocity (e') was determined by spectral tissue Doppler imaging (GE Vivid i) using standard methods. The e' velocity is inversely related to the time constant of isovolumic relaxation (τ), derived from $\tau = (14.70 - 100e')/0.15$ [19]. Early (E) and late (A) transmitral flow velocities, the isovolumetric relaxation time (IVRT), and the deceleration time of early filling velocity (Dec T) were measured by pulsed-wave Doppler (GE Vivid i). End-diastolic pressure was estimated as $EDP = 11.96 + 0.596 \times E/e'$ [19].

Left Ventricular Volumes and Contractility: In the upright seated rest position and during exercise, LV end diastolic (EDV), and end-systolic (ESV) volumes, along with ejection fraction (EF) were determined from Simpson's biplane method; the recommended method for measurement of volumes [18]. Cardiac index (Ci) was determined from the product of heart rate (HR) and stroke volume index. Load-independent measures of chamber contractility were examined as: 1) pre-load recruitable stroke work (PRSW [calculated from product of peak volumetric ejection rate from LV outflow Doppler and systolic BP, divided by EDV]), determined from the validated single-beat technique [20] and; 2) LV end-systolic elastance (E_{es} [calculated from BP, stroke volume, EF, and pre-ejection and systolic ejection time intervals from LV outflow Doppler]), determined by the validated single-beat technique [21]. The orientation of the LV outflow Doppler velocity was positioned 5 mm proximal to the aortic valve in the apical five

chamber view. The change in each parameter from upright seated rest to peak exercise was used to characterize contractile reserve.

Arterial Function: Effective arterial elastance (E_a), a measure of the net arterial load, was calculated as end-systolic pressure (ESP)/stroke volume (SV), where ESP is approximated as $0.9 \times$ systolic blood pressure (SBP) [9]. Systemic vascular resistance index (SVRI) was calculated as mean arterial pressure (MAP) $\times$ 80/Ci. The change in each parameter from upright seated rest to peak exercise was used to characterize global arterial reserve.

Arterial-Ventricular Coupling: The LV and the central arteries have bidirectional interactions. These interactions were examined the depiction of the function of the LV and arterial system in terms of elastance i.e., LV chamber elastance (E_{es}) and arterial Elastance (E_a), and examining their ratio (arterial-ventricular coupling ratio: E_a/E_{es}) [9].

Scaling for Body Size

Adequate scaling of physiological measures for body size is essential for correct interpretation, and often the relationship between body size and physiological function may not be linear, a major assumption for the ratiometric scaling approach [22]. The allometric scaling approach accounts for this potential nonlinear relationship by normalizing physiological measures using exponential powers that linearize the relationship. In our data set, the relationship between

body surface area (BSA) and cardiac volumes, cardiac output, and PRSW were adequately scaled for using the ratiometric scaling approach. No relationships were presented between BSA and Ees, or Ea. To account for differences in chamber size, Ees was normalized to end-diastolic volume (EDV). However, EDV was allometrically related to Ees and thus EDV to the power of 0.45 was used to scale Ees. Also, LVM was allometrically scaled to height to the power of 2.7. The relationship between BSA and the CV parameters were examined using Pearson's correlation. Initially, significant correlations were adjusted ratiometrically, $y = a + bx + \varepsilon$, where b represents the slope of the line of best fit, a the intercept on the y -axis, and ε the additive residual error term. If significant correlations remained between the ratiometrically adjusted CV variable and BSA, then these data were adjusted allometrically, $y = ax^b\varepsilon$, where y represents the CV variable, x represents BSA, a represents the proportionality coefficient or constant multiplier, b is the power function exponent, and ε represents the multiplicative residual error term, as described in detail previously [22].

Sample Size and Statistical Analysis

Measurements of CV function were performed offline, by a single investigator who was blinded to group allocation. The intra-class correlation coefficient (ICC) for all echocardiographic variables was derived in a subset of subjects ($n=8$). At rest, the ICC for all variables, collected on two separate days, was >0.80 . Similar results were obtained for echocardiographic variables

evaluated during peak exercise with all variables having an ICC>0.80 with the exception of the arterial-ventricular coupling ratio (ICC=0.63).

Based on previous data identifying differences in peak exercise Ees (≈ 4 mmHg/ml/m²) and the Ea/Ees ratio (≈ 0.10) between young and older healthy individuals [23-25] sample size was calculated as follows using G*Power 3.1: Assuming independence among subjects and a SD estimate of 6.5 mmHg/ml/m² for peak Ees and 0.11 for the Ea/Ees ratio at peak exercise, eighteen subjects per group would provide us with an 80 percent power (2-sided $\alpha=0.05$), to detect a difference of 4 mmHg/ml/m² for peak Ees and 0.10 for peak Ea/Ees between any two groups.

All analyses were performed using SPSS version 20 (SPSS Inc, Chicago, Illinois). A two-tailed $p \leq 0.05$ was required for significance. Data are reported as mean $\pm$ SEM unless otherwise stated. Normality was evaluated by the Kolmogorov-Smirnov test. Categorical variables were compared by the chi-square test. Continuous variables were log transformed as necessary and compared between groups through ANCOVA adjusting for sex. The changes from rest to peak exercise in CV parameters were examined by: a) calculating their delta and comparing this variable between groups using ANCOVA adjusting for sex, and; b) by examining a time (seated rest to peak exercise) by group (MetS vs. healthy controls) interaction evaluated using a two-way repeated-measures ANOVA. Further, to account for structural differences (LV Mass, cfPWV, cIMT) between groups we: c) examined the time by group interaction adjusting for LV Mass, cfPWV, and cIMT as covariates in the repeated-measures

ANCOVA; d) in a subset of the population, individuals (MetS vs. controls) were matched by age, LV Mass, cfPWV, cIMT, peak HR, and respiratory exchange ratio, and analyzed using a repeated-measures ANCOVA (time by group interaction) (see supplemental data).

Results

Subject Characteristics

As expected, baseline CVD risk factors were significantly different between MetS and controls but not for age or sex as summarized in Table 1.

Cardiovascular Differences at Rest

Arterial Structure and Function

In MetS subjects, carotid diameter, carotid CSA and cIMT were larger ($p<0.05$) compared to controls. As such, carotid circumferential stress was larger in MetS (Table 2). Although net arterial load (E_a) and vascular resistance (SVR_i) did not significantly differ between groups (Table 3), cfPWV was moderately increased (23%, $p<0.01$) in MetS vs. controls and remained significant after adjusting for MAP between groups ($p=0.01$), indicating an increase in arterial stiffness in MetS.

Left Ventricular Structure and Function

LVmass was 37 percent larger in MetS, but RWT was similar compared to controls. Thus, LVH was 18 percent greater in MetS than controls, with 33 percent of MetS presenting with concentric remodeling and 7 percent presenting with concentric and eccentric LVH (Table 2). In addition to these remodeling differences between groups; the MetS group tended to present with reduced supine LV diastolic function evident by a greater impaired LV relaxation (lower e' and longer τ), a higher A-wave (thus the E/A ratio was reversed $p<0.01$), and a

higher E/e' (a predictor of elevated LV filling pressure), and EDP vs. controls (Table 3).

Comparisons of EDV, ESV, and SV were significantly elevated in MetS however, after adjusting to BSA, cardiac volume no longer differed between groups (Table 4). Cardiac index (Ci) and HR were also similar between groups. LV load-independent contractility as assessed by Ees, $Ees \div EDV^{0.45}$, and PRSW did not differ between MetS and controls at rest. The similarity between groups in Ees and Ea guaranteed that Ea/Ees (a measure of net cardiovascular performance) was also comparable between groups.

Cardiovascular Responses to Upright Exercise

Exercise Performance and Cardiac Function

Aerobic capacity adjusted to body weight (ml/min/kg) at peak exercise and at the ventilatory threshold were ($p < 0.001$) lower in MetS than healthy controls, this was despite both MetS and controls achieving similar rates of perceived exercise exhaustion, exercise duration and peak workload (Table 5).

During exercise, there was no significant time by group interactions for components of LV diastolic function, namely, IVRT, E-wave, A-wave, and Dec T (Figure 2). The change from rest to peak exercise in EDVi ($p > 0.9$) and SVi ($p = 0.3$) did not significantly differ between MetS and controls. However, there was a significant time by group interaction for the reduction in ESVi during exercise with MetS individuals having a blunted decrease vs. healthy controls (Figure 3). MetS individuals also demonstrated a blunted increase in EF (6%,

$p=0.02$), and C_i (-24%, $p<0.05$) compared to controls, which were evident at submaximal workloads (Figure 3); whereas peak HR was lower at peak exercise in MetS. Importantly, LV contractility reserve (change from rest to peak exercise) was impaired in MetS compared to controls, evident by a blunted increase in E_{es} (-35%, $p=0.01$), and $PRSW_i$ (-26%, $p=0.013$) in MetS (Figure 4). Once again these differences were manifested at submaximal exercise workloads. Further, even after adjusting statistically the above parameters individually for either peak HR, LV Mass, $cIMT$, or $cfPWV$ the results above did not differ significantly. Further, in a subcohort of this population matched for resting LV Mass, $cIMT$, $cfPWV$, peak exercise HR and respiratory exchange ratio, the results above did not differ significantly (see supplemental data).

Arterial Function and AVC Reserve

During exercise the change in E_a did not differ between groups ($p>0.2$) (Figure 4B). The combination of an impaired contractile reserve in MetS, but a similar change in E_a between groups during exercise considerably limited the E_a/E_{es} response in MetS (25% impaired decrease, $p<0.05$) compared to controls (Figure 4A). Submaximal and peak SBP and DBP were higher in MetS than controls (Figure 5A), and a significant time by group interaction was noted for the change in SVR_i during exercise. We further examined these data by statistically adjusting the above CV variables for LV Mass, $cIMT$, or $cfPWV$, and with the exception of SVR_i ($p=0.1$ time by group interaction), the results above did not

differ significantly. Similarly, the results above did not differ significantly from the subcohort analysis (see supplemental data).

Discussion

The present study provides the first comparison of LV and arterial structure and function responses to exercise in non-diabetic MetS individuals without overt CVD. This finding is of significant clinical interest, and a growing public health concern. Our study provides evidence of LV systolic dysfunction during exercise including limitations in peripheral vasodilation accumulating in a blunted arterial-ventricular coupling reserve and impaired peak aerobic capacity in MetS. These data demonstrate that pathophysiological CV alterations occur in the earliest stages of MetS development, prior to any evidence of chronic disease such as diabetes and/or overt CVD, and that impaired LV systolic function during exercise occurs prior to evidence of LV systolic dysfunction at rest.

Resting LV and Arterial Structure and Function

Previous studies examining CV alterations in MetS patients (with or without diabetes) have been limited to characterizing differences in LV and arterial structure and function at rest. MetS patients are traditionally characterized as having increased cIMT (range 9-16%), and PWV (range 13-32%) [26, 27]. The results of the present study are similar to previous findings with MetS individuals presenting with a 21 percent higher PWV, and an 18 percent higher cIMT. They also confirm the presence of increased LV Mass in MetS (in the absence of CVD and/or diabetes) reported by existing studies [7, 27].

Although the LV structural differences in MetS are fairly well agreed upon, consensus is lacking with regard to changes in LV function. Some studies [3-8], but not all [7, 8], have suggested that MetS individuals have LV systolic dysfunction at rest [3-6]. However, many of these studies used EF as a measure of systolic function, which despite its conventional clinical application, is a rather poor prognostic measure of systolic function as it is potentially influenced by loading conditions and chamber remodeling [28]. Further, the populations examined in these studies were confounded by the inclusion of MetS patients with diabetes [4, 7, 11] and moderate stenosis [5], conditions that would exacerbate symptoms of LV dysfunction. In addition, control subjects included people with hypertension [4-6], obesity [7], diabetes [3, 4, 11], and many were taking CV medications [5, 11]. Such confounding factors do not allow interpretation as to whether the changes in LV function are representative of MetS or of other existing pathologies. Using load-independent measures of contractility (E_{es} , or $E_{es} \cdot EDV$, and PRSWi) we found no evidence of LV dysfunction in MetS. The present findings cannot exclude the possibility of cellular abnormalities in the cardiomyocytes of MetS individuals. However, we did identify differences in resting diastolic function in MetS versus healthy controls (Table 4), which has been previously reported in some [6, 8] but not all [29, 30] studies. It has been postulated that LV diastolic dysfunction is a pre-cursor of LV systolic dysfunction and heart failure with a preserved EF (HFpEF) [31-33]. In addition, abnormalities of LV relaxation, i.e. grade 1 diastolic dysfunction, confer a two-fold increase in all-cause and cardiac mortality [32]. This statistic highlights

the clinical importance of recognizing the early, and/or subclinical, changes in diastolic function at rest in individuals with MetS.

Aerobic Capacity and Cardiovascular Reserve Function

Exercise provides a powerful tool to examine the response of the LV and arterial systems to stress and to assess functional reserve. If LV function is impaired in MetS individuals it would likely be revealed during exercise, and to promote intolerance to exercise.

Widely regarded as a load-independent measure of LV chamber performance, the change from rest to peak exercise in Ees is blunted with advancing age and may be limited further in the presence of disease [34]. Although at rest Ees is determined by geometric and biochemical properties that regulate LV end-systolic stiffness (i.e. structural changes from LV hypertrophy or fibrosis) [35], acute changes in Ees, such as those observed during exercise reflect inherent changes in LV contractile function [9]. Using load-independent measures of LV contractility, we identified a blunted Ees, and PRSWi reserve capacity in MetS that manifested at submaximal workloads (Figure 4). Ha et al. [36] noted a blunted increase in s' in MetS during exercise; however the extent of the LV function impairment was limited by the load dependence of s' and the lack of a healthy control for comparison. In the present study, impaired LV contractility was accompanied by altered cardiac pump performance, and an impaired vasodilator reserve capacity in MetS. Importantly, impaired contractile function and vasodilator capacity resulted in a blunted arterial-ventricular coupling

response to exercise. Ea/Ees is a key determinant of CV performance, cardiac energetics and exercise capacity [9]. At rest in healthy individuals, Ea/Ees varies from 0.5-1.0 to ensure maximal cardiac power and chamber efficiency [12]. During exercise, Ea/Ees decreases due to an acute mismatch between Ees and Ea to optimize cardiac performance [9]. While resting Ea/Ees did not differ between our groups, the Ea/Ees response to exercise was considerably blunted, which in part contributed to reduced peak aerobic capacity in MetS. Similar impairments in Ea/Ees have been reported during acute maximal exercise with advancing age and have been suggested to explain, in part, diminished CV functional capacity in the elderly [34].

Mechanisms limiting CV reserve in MetS individuals remain speculative. But, it is likely that multiple factors are involved, including sympathetic nervous system activity, activation of the renin-angiotensin-aldosterone system, and cardiac metabolism during exercise [37-39]. Importantly, the blunted CV response is unlikely to be attributed to differences in exercise effort exercise duration, maximal workload, and peak BORG were similar between groups (Table 5). Further, the blunted CV responses in MetS remained evident even after: a) adjusting statistically for peak HR and the respiratory exchange ratio (which were higher in MetS vs. controls); and b) in the subgroup analyses in which subjects were matched for peak HR and the respiratory exchange ratio. Impairments in contractile reserve observed in the present study may be mediated in part by altered calcium handling, as the influx of calcium is a primary determinant of cardiac performance. Specifically, contractile dysfunction as a

result of alterations in sarcoplasmic reticulum calcium handling has been implicated in aging and type 2 diabetes [40, 41]. Although there was no sign of wall motion abnormalities during exercise in MetS, the use of upright echocardiography may have missed subtle wall motion abnormalities [42]. Thus this blunted systolic function in MetS could, in part, be a manifestation of early myocardial ischemia even in the presence of normal wall motion during exercise.

An additional potential mechanism for the impaired CV response to exercise in MetS may be depressed systemic peripheral vasodilation in the resistance vessels. An impaired SVR_i response to exercise is evident in HFpEF and is known to contribute to limited exercise capacity in systolic heart failure, and is thought to be a result of a reduction in NO generation [43, 44]. In accordance with these findings, we observed a blunted reduction in SVR_i during exercise in MetS vs. controls. Indeed, endothelial dysfunction is a common denominator linking MetS, type 2 diabetes, and CVD [45] suggesting that therapy targeted to promoting the bioavailability and/or stimulating the generation of NO may effectively improve systemic vasodilatation during exercise in MetS.

The MetS is characterized by structural changes to the heart (increase LV Mass and remodeling) and conduit arteries (increased cIMT and cfPWV) [27, 46]. These structural changes are not only predictors of CV events (myocardial or cerebral infarction) [47, 48] and mortality [49, 50] but they are also predictors of poor aerobic capacity [51-54]. It is plausible that the LV and arterial structural differences noted between MetS and healthy controls could contribute to the blunted CV response during exercise. However, after adjusting for LV mass,

cIMT, or cfPWV in the statistical models, the blunted Ees, PRSWi, Ci and EF response from rest to peak exercise in MetS remained. We further examined these relationships by matching healthy controls and MetS by LV mass, cIMT, and cfPWV. Once again, MetS individuals demonstrated a blunted CV response to exercise compared to healthy controls in this subcohort. These results suggest that the structural CV differences between MetS and healthy controls do not fully account for the impaired exercise CV response in MetS.

Previous literature has suggested a link between LV diastolic dysfunction and exercise intolerance [7, 55]. Although the focus of this study was to examine the extent of LV systolic dysfunction during exercise (and thus echocardiographic views were optimized to examine systolic function), we were able to compare some components of LV diastolic function during exercise between MetS and controls. No differences in IVRT, and Dec T were evident between groups. Further, the early (E) and late (A) mitral inflow filling velocities, during submaximal workloads, did not differ between groups. At moderate exercise workloads there is a high incidence of fusion of the E and A-wave, thus from 50 W onwards the A-wave dominated and no differences were noted between groups at peak exercise (Figure 2). Unfortunately the acquisition of LV diastolic parameters during exercise is cumbersome, limiting our ability to fully characterize the extent of exercise diastolic function. Therefore we cannot eliminate the possibility that diastolic abnormalities during exercise may have contributed to the CV reserve deficits.

Limitations

Due to the small sample size and cross-sectional nature of our study, we cannot infer causality between MetS and CV abnormalities. Although pressure and flow were not directly measured, but rather estimated from non-invasive surrogates, these have been previously validated against invasive hemodynamic measurements performed at rest [20, 21]. However, the non-invasive measurement of end-systolic elastance and pre-load recruitable stroke work have been validated during exercise. Further, our peak cardiac data may be underestimated due to the challenge of acquiring echocardiographic images during exercise, but this technique has been successfully used by others [56, 57], to which we observe similar values and responses in our subjects, suggesting fidelity in our data. Although, it is possible that the lower peak HR and respiratory exchange ratio in MetS may have contributed to their blunted CV response to exercise, this impaired CV response remained evident in a subcohort of individuals matched for peak HR and the respiratory exchange. The overall significance and strength of our study is that our MetS patient population did not have the disease-related confounders and/or medications seen in most of the existing studies, which allows to report a comprehensive examination of resting and exercise arterial and LV measures in MetS individuals prior to the development of chronic disease.

Conclusion

This study demonstrates that individuals with MetS display evidence of impaired systolic contractile function, vasodilator, and cardiac pump function

reserve capacity during exercise. These deficits contribute to abnormal arterial-ventricular coupling and exercise intolerance in individuals with MetS without diabetes and/or overt CVD. Whether the development of MetS and associated CV changes, and the progression to MetS together with diabetes is along the same pathophysiological pathway to heart failure warrants further investigation.

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Reference List

1. Malik, S., et al., *Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults*. Circulation, 2004. 110(10): p. 1245-1250.
2. Mozumdar, A. and G. Liguori, *Persistent Increase of Prevalence of Metabolic Syndrome Among U.S. Adults: NHANES III to NHANES 1999–2006*. Diabetes Care, 2011. 34(1): p. 216-219.
3. Azevedo, A., et al., *Increasing number of components of the metabolic syndrome and cardiac structural and functional abnormalities - cross-sectional study of the general population*. BMC Cardiovascular Disorders, 2007. 7(1): p. 17.
4. Aijaz, B., et al., *Abnormal Cardiac Structure and Function in the Metabolic Syndrome: A Population-Based Study*. Mayo Clinic Proceedings, 2008. 83(12): p. 1350-1357.
5. Pagé, A., et al., *Metabolic Syndrome Is Associated With More Pronounced Impairment of Left Ventricle Geometry and Function in Patients With Calcific Aortic Stenosis: A Substudy of the ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin)*. Journal of the American College of Cardiology, 2010. 55(17): p. 1867-1874.
6. Mahmud, A., et al., *Left ventricular structural and functional changes in the metabolic syndrome*. J Cardiometab Syndr, 2009. 4(2): p. 81-8.

7. de las Fuentes, L., et al., *Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass*. Eur Heart J, 2007. 28(5): p. 553-9.
8. Chinali, M., et al., *Comparison of cardiac structure and function in American Indians with and without the metabolic syndrome (the Strong Heart Study)*. Am J Cardiol, 2004. 93(1): p. 40-4.
9. Chantler, P.D., E.G. Lakatta, and S.S. Najjar, *Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise*. Journal of Applied Physiology, 2008. 105(4): p. 1342-1351.
10. Gong, H.P., et al., *Impaired left ventricular systolic and diastolic function in patients with metabolic syndrome as assessed by strain and strain rate imaging*. Diabetes Res Clin Pract, 2009. 83(3): p. 300-7.
11. Wong, C.Y., et al., *Myocardial and vascular dysfunction and exercise capacity in the metabolic syndrome*. Am J Cardiol, 2005. 96(12): p. 1686-91.
12. De Tombe, P.P., et al., *Ventricular stroke work and efficiency both remain nearly optimal despite altered vascular loading*. Am J Physiol, 1993. 264(6 Pt 2): p. H1817-24.
13. Fletcher, G.F., et al., *Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association*. Circulation, 2001. 104(14): p. 1694-740.
14. Huang, P., *A Comprehensive Definition for Metabolic Syndrome*. Disease Models & Mechanisms, 2009. 2(2): p. 231-237.

15. Borg, G.A., *Perceived exertion*. Exerc Sport Sci Rev, 1974. 2: p. 131-53.
16. Roman, M.J., et al., *American Society of Echocardiography Report: Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society for Vascular Medicine and Biology*. Vascular Medicine, 2006. 11(3): p. 201-211.
17. O'Rourke, M.F., A. Pauca, and X.J. Jiang, *Pulse wave analysis*. Br J Clin Pharmacol, 2001. 51(6): p. 507-22.
18. Lang, R.M., et al., *Recommendations for chamber quantification*. Eur J Echocardiogr, 2006. 7(2): p. 79-108.
19. Ommen, S.R., et al., *Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study*. Circulation, 2000. 102(15): p. 1788-94.
20. Lee, W.S., et al., *Estimation of preload recruitable stroke work relationship by a single-beat technique in humans*. Am J Physiol Heart Circ Physiol, 2003. 284(2): p. H744-50.
21. Chen, C.H., et al., *Noninvasive single-beat determination of left ventricular end-systolic elastance in humans*. J Am Coll Cardiol, 2001. 38(7): p. 2028-34.
22. Chantler, P.D., et al., *The influence of body size on measurements of overall cardiac function*. Am J Physiol Heart Circ Physiol, 2005. 289(5): p. H2059-65.

23. Chantler, P.D., et al., *The sex-specific impact of systolic hypertension and systolic blood pressure on arterial-ventricular coupling at rest and during exercise*. American Journal of Physiology-Heart and Circulatory Physiology, 2008. 295(1): p. H145-H153.
24. Chantler, P., et al., *Attenuating The Age-associated Deficit In Peak Arterial-ventricular Coupling With Sodium Nitroprusside: 2450: Board #58 June 4 9:00 AM - 10:30 AM*. Medicine & Science in Sports & Exercise, 2010. 42(5): p. 630 10.1249/01.MSS.0000385756.72940.ee.
25. Najjar, S.S., et al., *Age and gender affect ventricular-vascular coupling during aerobic exercise*. J Am Coll Cardiol, 2004. 44(3): p. 611-7.
26. Lin, H.-F., et al., *The risk of the metabolic syndrome on carotid thickness and stiffness: Sex and age specific effects*. Atherosclerosis, 2010. 210(1): p. 155-159.
27. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. 43(8): p. 1388-95.
28. Kass, D.A., et al., *Comparative influence of load versus inotropic states on indexes of ventricular contractility: experimental and theoretical analysis based on pressure-volume relationships*. Circulation, 1987. 76(6): p. 1422-36.
29. Cuspidi, C., et al., *Metabolic syndrome and target organ damage in untreated essential hypertensives*. J Hypertens, 2004. 22(10): p. 1991-8.

30. Schillaci, G., et al., *Different Impact of the Metabolic Syndrome on Left Ventricular Structure and Function in Hypertensive Men and Women*. Hypertension, 2006. 47(5): p. 881-886.
31. Schannwell, C.M., et al., *Left ventricular diastolic dysfunction as an early manifestation of diabetic cardiomyopathy*. Cardiology, 2002. 98(1-2): p. 33-9.
32. Bella, J.N., et al., *Mitral ratio of peak early to late diastolic filling velocity as a predictor of mortality in middle-aged and elderly adults: the Strong Heart Study*. Circulation, 2002. 105(16): p. 1928-33.
33. Kitzman, D.W., et al., *Importance of heart failure with preserved systolic function in patients > or = 65 years of age. CHS Research Group. Cardiovascular Health Study*. Am J Cardiol, 2001. 87(4): p. 413-9.
34. Chantler, P.D. and E. Lakatta, *Arterial-Ventricular Uncoupling with Age and Disease*. Frontiers in Physiology, 2012. 3.
35. Borlaug, B.A. and D.A. Kass, *Ventricular-vascular interaction in heart failure*. Cardiol Clin, 2011. 29(3): p. 447-59.
36. Ha, T.H., et al., *The Effect of Metabolic Syndrome on Myocardial Contractile Reserve during Exercise in Non-Diabetic Hypertensive Subjects*. J Cardiovasc Ultrasound, 2011. 19(4): p. 176-82.
37. Engeli, S., R. Negrel, and A.M. Sharma, *Physiology and pathophysiology of the adipose tissue renin-angiotensin system*. Hypertension, 2000. 35(6): p. 1270-7.

38. Peterson, L.R., et al., *Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging*. J Am Coll Cardiol, 2004. 43(8): p. 1399-404.
39. Turhan, H., et al., *Impaired coronary blood flow in patients with metabolic syndrome: documented by Thrombolysis in Myocardial Infarction (TIMI) frame count method*. Am Heart J, 2004. 148(5): p. 789-94.
40. Lakatta, E.G. and S.J. Sollott, *Perspectives on mammalian cardiovascular aging: humans to molecules*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2002. 132(4): p. 699-721.
41. Poirier, P., et al., *Diastolic Dysfunction in Normotensive Men with Well-Controlled Type 2 Diabetes: Importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy*. Diabetes Care, 2001. 24(1): p. 5-10.
42. Ryan, T., et al., *Detection of coronary artery disease with upright bicycle exercise echocardiography*. J Am Soc Echocardiogr, 1993. 6(2): p. 186-97.
43. Abudiab, M.M., et al., *Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction*. Eur J Heart Fail, 2013.
44. Borlaug, B.A., et al., *Impaired Chronotropic and Vasodilator Reserves Limit Exercise Capacity in Patients With Heart Failure and a Preserved Ejection Fraction*. Circulation, 2006. 114(20): p. 2138-2147.

45. Diamant, M. and M.E. Tushuizen, *The metabolic syndrome and endothelial dysfunction: common highway to type 2 diabetes and CVD*. Curr Diab Rep, 2006. 6(4): p. 279-86.
46. Scuteri, A., et al., *The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study*. European Heart Journal, 2010. 31(5): p. 602-613.
47. Laurent, S., et al., *Aortic Stiffness Is an Independent Predictor of Fatal Stroke in Essential Hypertension*. Stroke, 2003. 34(5): p. 1203-1206.
48. Protopsaltis, J., et al., *Prediction of long-term functional outcome in patients with acute ischemic non-embolic stroke*. Atherosclerosis, 2009. 203(1): p. 228-35.
49. Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis, *Prediction of Cardiovascular Events and All-Cause Mortality With Arterial Stiffness: A Systematic Review and Meta-Analysis*. Journal of the American College of Cardiology, 2010. 55(13): p. 1318-1327.
50. Stevens, S.M., K. Reinier, and S.S. Chugh, *Increased Left Ventricular Mass as a Predictor of Sudden Cardiac Death: Is it Time to Put it to The Test?* Circulation: Arrhythmia and Electrophysiology, 2013. 6(1): p. 212-217.
51. Hundley, W.G., et al., *Cardiac cycle-dependent changes in aortic area and distensibility are reduced in older patients with isolated diastolic heart failure and correlate with exercise intolerance*. J Am Coll Cardiol, 2001. 38(3): p. 796-802.

52. Jae, S.Y., et al., *Association between cardiorespiratory fitness and arterial stiffness in men with the metabolic syndrome*. Diabetes Res Clin Pract, 2010. 90(3): p. 326-32.
53. Jae, S.Y., et al., *Cardiorespiratory fitness and carotid artery intima media thickness in men with type 2 diabetes*. J Phys Act Health, 2012. 9(4): p. 549-53.
54. Kokkinos, P., et al., *Exercise Capacity and Blood Pressure Associations With Left Ventricular Mass in Prehypertensive Individuals*. Hypertension, 2007. 49(1): p. 55-61.
55. Poirier, P., et al., *Impact of left ventricular diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 diabetes mellitus*. Am J Cardiol, 2000. 85(4): p. 473-7.
56. Borlaug, B.A., et al., *Global Cardiovascular Reserve Dysfunction in Heart Failure With Preserved Ejection Fraction*. Journal of the American College of Cardiology, 2010. 56(11): p. 845-854.
57. Tartière-Kesri, L., et al., *Increased Proximal Arterial Stiffness and Cardiac Response With Moderate Exercise in Patients With Heart Failure and Preserved Ejection Fraction*. Journal of the American College of Cardiology, 2012. 59(5): p. 455-461.

Table 1. Clinical Characteristics

	Controls (n=20)	MetS (n=27)	p Value
Age, years	45 ± 2.5	49 ± 1.9	0.24
Sex, female %	65	63	0.57
Height, cm	169 ± 2	169 ± 2	0.92
Weight, kg	71 ± 2	103 ± 4	<0.001
Waist circumference, cm	85 ± 5	119 ± 5	<0.001
Body Fat, %	25 ± 2	39 ± 2	<0.001
BSA, m ²	1.80 ± 0.03	2.11 ± 0.04	<0.001
BMI, kg/m ²	25.0 ± 0.8	36.0 ± 1.0	<0.001
SBP, mmHg*	115 ± 2	134 ± 3	<0.001
DBP, mmHg*	75 ± 1	83 ± 1	<0.001
Ejection Fraction, %	61 ± 1	58 ± 1	0.06
Triglycerides, mg/dL	94 ± 10	170 ± 29	0.036
HDL, mg/dL	55 ± 3	43 ± 2	<0.001
Glucose, mg/dL	93 ± 2	99 ± 1	0.018
HbA1c, %	5.35 ± 0.09	5.60 ± 0.07	0.03
Insulin, µIU/mL	6.1 ± 1	12.1 ± 1	<0.001
HOMA-IR	1.32 ± 0.23	2.98 ± 0.37	<0.001
Hypertensive %	0	56 %	<0.01
Diabetes Mellitus %	0	0	
Medications			
Hypertension	0	56%	<0.05
Cholesterol	0	7%	<0.05

Values are mean ± 1 SEM; BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoprotein; HbA1c: hemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance. *SBP and DBP taken during screening visit

Table 2: Measures of Cardiovascular Geometry at Supine Rest

	Controls (n=20)			MetS (n=27)			p Value
Arterial Geometry							
clMT, mm	0.61	±	0.02	0.72	±	0.04	0.02
Diastolic diameter, mm	5.78	±	0.12	6.17	±	0.13	0.04
Carotid CSA, mm ² /m ²	10.3	±	0.32	12.7	±	0.68	<0.01
Cardiac Geometry							
LV Mass, g	136	±	7	186	±	11	<0.01
LV Mass Index, g/m ²	75	±	3	88	±	5	0.046
LV Mass/height ^{2.7} , g/m ^{2.7}	33	±	1	46	±	3	<0.01
Relative Wall Thickness	0.39	±	0.02	0.41	±	0.02	0.41
LV Hypertrophy %		1			19		0.18
Concentric Remodeling, %		25			33		0.14
Concentric Hypertrophy, %		1			7		0.14
Eccentric Hypertrophy, %		0			7		0.14
Values are mean ± 1 SEM, p-values adjusted for sex; clMT: carotid intima-medial thickness; CSA: cross-sectional area.							

Table 3: Measures of Left Ventricular Diastolic Function at Supine Rest

	Controls (n=20)			MetS (n=27)			p Value
LV Diastolic Function							
E, m/s	0.81	±	0.04	0.82	±	0.03	0.65
A, m/s	0.57	±	0.03	0.73	±	0.03	<0.01
E/A ratio	1.47	±	0.08	1.19	±	0.07	<0.01
IVRT, m/s	71	±	3	74	±	3	0.54
Dec T, m/s	220	±	7	203	±	7	0.10
e', m/s	0.13	±	0.01	0.11	±	0.01	0.03
τ, ms	28	±	3	41	±	3	<0.01
E/e'	6.46	±	0.40	8.19	±	0.59	0.02
LV EDP, mmHg	15.9	±	0.24	16.9	±	0.36	0.02
Values are mean ± 1 SEM, p-values adjusted for sex; E: peak velocity of the early diastolic mitral flow; A: peak velocity of the late diastolic mitral flow; E/A: E divided by A; IVRT: isovolumetric relaxation time; Dec T: mitral flow deceleration time of early filling velocity; e': mitral annular early diastolic velocity; τ: time constant of isovolumetric relaxation; E/e': E divided by e'; LV EDP: left ventricular end-diastolic pressure							

Table 4: Measures of Cardiovascular Function at Seated Rest

	Controls (n=20)	MetS (n=27)	p Value
LV Performance			
EDV, ml	87 ± 6	103 ± 5	0.02
EDVi, ml/m ²	48 ± 3	48 ± 2	0.92
ESV, ml	36 ± 3	49 ± 2	<0.01
ESVi, ml/m ²	20 ± 1	21 ± 1	0.52
SV, ml	51 ± 3	59 ± 3	0.09
SVi, ml/m ²	28 ± 2	28 ± 1	0.76
LV Contractility			
Ees, mmHg/ml	3.21 ± 0.33	2.91 ± 0.18	0.45
Ees·EDV ^{0.45}	85 ± 6	91 ± 25	0.48
PRSW, g/cm ²	90 ± 4	94 ± 3	0.93
PRSWi, g/cm ² /m ²	52 ± 2	51 ± 2	0.66
Integrated indexes			
Heart rate, bpm	68 ± 2	66 ± 2	0.46
Ea/Ees ratio	0.91 ± 0.06	0.93 ± 0.07	0.93
Cardiac Output	3.43 ± 0.22	3.85 ± 0.20	0.18
Cardiac Index, L/m ² ·min	2.03 ± 0.12	2.16 ± 0.08	0.33
Arterial Function			
SBP, mmHg	116 ± 3	127 ± 3	<0.01
MAP, mmHg	90 ± 2	99 ± 2	<0.01
Ea, mmHg/ml	2.71 ± 0.17	2.58 ± 0.15	0.58
SVRi, dyne·m ² /s·cm ⁻⁵	1261 ± 78	1063 ± 75	0.08
cf-PWV, m/s	6.6 ± 0.2	8.0 ± 0.2	<0.01 [≠]
Carotid Circum Stress, mmHg	18.8 ± 0.8	23.4 ± 1.4	0.01
Values are mean ± 1 SEM, p-values adjusted for sex; [≠] adjusted for differences in mean arterial pressure; EDV: end-diastolic volume (i=index); SV: stroke volume; Ees: end-systolic elastance; PRSWi: preload recruitable stroke work index; Ea/Ees: arterial-ventricular coupling ratio; Ea: arterial elastance; SVRi: systemic vascular resistance index; cf-PWV: carotid to femoral pulse wave velocity.			

Table 5: Metabolic Performance during Exercise

	Controls (n=20)	MetS (n=27)	p Value
Exercise duration, s	869 ± 65	946 ± 49	0.34
Peak respiratory exchange ratio	1.15 ± 0.02	1.09 ± 0.02	0.01
Peak work load, W	113 ± 9	123 ± 5	0.29
Peak Heart rate, bpm	166 ± 4	154 ± 3	0.01
Peak SBP, mmHg	177 ± 8	191 ± 4	0.01
Peak DBP, mmHg	68 ± 4	77 ± 4	0.09
Subjective effort score (6-20)	19 ± 0.3	19 ± 0.3	0.56
VO _{2peak} , ml/kg/min	24.6 ± 1.4	17.1 ± 0.9	<0.01
VO ₂ at ventilatory threshold, ml/kg/min	16.8 ± 1.1	11.8 ± 0.8	<0.01
Time to ventilatory threshold, s	638 ± 72	628 ± 57	0.91
Ratio VO ₂ at ventilatory threshold to VO _{2peak} , %	65 ± 2	67 ± 2	0.58
Ratio of observed to predicted VO _{2peak} , %	91 ± 5	69 ± 2	<0.01
VE/VO ₂ at VT, %	29 ± 1	29 ± 1	0.70
VE/VO ₂ at max	41 ± 1	38 ± 1	0.10
VE/VCO ₂ at max	35 ± 1	35 ± 1	0.75
Values are mean ± 1 SEM, p-values adjusted for sex			

Figure Legends

Figure 1. Schematic illustration of the experimental set-up for the graded exercise test. Subjects performed maximal upright cycle ergometry with the back support angled at approximately 130°.

Figure 2. LV Diastolic function at rest and during exercise. The change from rest to max exercise in (A) isovolumetric relaxation time (IVRT), (B) early (E) and late (A) mitral inflow velocities, and (C) the deceleration time of early filling velocity (Dec T) in MetS and controls. No significant time by group interactions for IVRT or Dec T were evident between MetS and controls during max exercise. Further at peak exercise no differences were observed between groups for the early (E) and late (A) mitral inflow filling velocities, however, at moderate workloads the E- and A-waves become superimposed at extreme workloads the A-wave dominates over the E-wave. * $p < 0.05$ MetS vs. controls; # $p < 0.05$ time by group interaction. Data presented as means $\pm$ SEM.

Figure 3. Cardiac pump performance at rest and during upright exercise. The change from rest to max exercise in (A) cardiac volumes, (B) ejection fraction (EF), (C) cardiac index (Ci), and (D) heart rate (HR) in MetS and controls. During exercise the change from rest to max exercise in EDVi and SVi was similar between MetS and healthy controls. A significant time by group interaction was identified for the reduction of ESVi during exercise with MetS displaying a blunted decrease vs. controls. Compared to controls MetS also demonstrated a blunted

increase in EF and Ci at max exercise, which was evident at submaximal workloads. Peak HR was significantly lower in MetS vs. controls. * $p < 0.05$ MetS vs. controls; # $p < 0.05$ time by group interaction. Data presented as means $\pm$ SEM.

Figure 4. Cardiac contractility and arterial-ventricular coupling response to exercise. The change from rest to max exercise in (A) pre-load recruitable stroke work index (PRSWi), (B) end-systolic elastance (Ees) and effective arterial-elastance (Ea), (C) and the arterial-ventricular coupling ratio (Ea/Ees) in MetS and controls. Compared to controls, MetS displayed a blunted increase in Ees and PRSWi at max exercise. During exercise the combination of a blunted increase in Ees and a comparable change in Ea between groups resulted in a blunted Ea/Ees ratio in MetS compared to controls. * $p < 0.05$ MetS vs. controls; # $p < 0.05$ time by group interaction. Data presented as means $\pm$ SEM.

Figure 5. Pressure and vascular resistance response during exercise. The change from rest to max exercise in (A) systolic (SBP) and diastolic (DBP) blood pressure, and (B) systemic vascular resistance index (SVRi) in MetS and controls. Systolic BP and diastolic BP were higher in MetS vs. controls at submaximal and maximal workloads. * $p = 0.08$ vs. controls; * $p < 0.05$ MetS vs. controls; # $p < 0.05$ time by group interaction. Data presented as means $\pm$ SEM.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Chapter 3

AEROBIC EXERCISE TRAINING REDUCES ARTERIAL STIFFNESS IN METABOLIC SYNDROME

*David A. Donley¹, *Sara B. Fournier^{1,2}, Brian L. Reger¹, Evan DeVallance^{1,2},
Daniel E. Bonner¹, I. Mark Olfert^{1,2}, Jefferson C. Frisbee^{2,3} and Paul D.
Chantler^{1,2}

*These authors contributed equally to the manuscript

¹Division of Exercise Physiology, School of Medicine, West Virginia University;

*²Center for Cardiovascular and Respiratory Sciences, School of Medicine, West
Virginia University; ³Department of Physiology and Pharmacology, School of
Medicine, West Virginia University.*

Running Head: Arterial stiffness and Metabolic Syndrome

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Abstract

The metabolic syndrome (MetS) is associated with a 3-fold increase risk of cardiovascular disease (CVD) mortality partly due to increased arterial stiffening. We compared the effects of aerobic exercise training on arterial stiffening/mechanics in MetS without overt CVD or Type 2 Diabetes. MetS and healthy controls (Con) underwent 8 weeks of exercise training (ExT; 11 MetS and 11 Con) or remained inactive (NonT; 11 MetS and 10 Con). The following measures were performed pre and post intervention: radial pulse wave analysis (applanation tonometry) was used to measure augmentation pressure and index, central pressures, and an estimate of myocardial efficiency; arterial stiffness was assessed from carotid-femoral pulse wave velocity (cfPWV, applanation tonometry); carotid thickness was assessed from B-mode ultrasound; and peak aerobic capacity (gas exchange) was performed in the seated position. Plasma matrix metalloproteinases (MMP), and CVD risk (Framingham risk score) were also assessed. cfPWV was reduced ($p<0.05$) in MetS-ExT (7.9 ± 0.6 to 7.2 ± 0.4 m/s) and Con-ExT (6.6 ± 1.8 to 5.6 ± 1.6 m/s). Exercise training reduced ($p<0.05$) central systolic pressure (116 ± 5 to 110 ± 4 , mmHg), augmentation pressure (9 ± 1 to 7 ± 1 , mmHg), augmentation index (19 ± 3 to $15\pm4\%$), and improved myocardial efficiency (155 ± 8 to 168 ± 9) but only in the MetS group. Aerobic capacity increased ($p<0.05$) in MetS-ExT (16.6 ± 1.0 to 19.9 ± 1.0) and Con-ExT (23.8 ± 1.6 to 26.3 ± 1.6). MMP-1 and 7 were correlated with cfPWV and both MMP-1 and 7 were reduced post-exercise training in MetS. These findings suggest that some

of the pathophysiological changes associated with MetS can be improved after aerobic exercise training thereby lowering their CV risk.

Keywords: metabolic syndrome, arterial stiffness, exercise training

Introduction

The metabolic syndrome (MetS) is associated with a 3-fold increase risk of cardiovascular disease (CVD) mortality than individuals without MetS [1]. Current predictions are that by the year 2020, as much as 40% of the population will have MetS. Part of this increased CVD risk in MetS is attributed to the pathophysiological changes to the arterial system, namely increased arterial stiffness, carotid thickening, and higher central arterial pressures [2, 3]. A 1 m/s increase in central pulse wave velocity (PWV: a measure of arterial stiffness) corresponds to an age-, sex-, and risk factor-adjusted risk increase of 15% in cardiovascular and all-cause mortality [4]. Further, a 0.1 mm increase in carotid artery intima media thickness (cIMT) is associated with a 18% and 15% increased risk for a stroke and myocardial infarction, respectively [5]. Therefore, it is critically important that effective interventions aimed at improving arterial health are identified.

Aerobic exercise training has been shown to be an effective intervention to improve arterial compliance/stiffness in healthy young and middle/older persons [6, 7]. However, with metabolic disease, previous studies have examined exercise interventions in populations with a single metabolic risk factor (obesity, hypertension, hypercholesterolemia) [8-10], or in MetS with type 2 diabetes (T2DM) [11, 12]. Because the development of MetS occurs prior to the development of T2DM and overt CVD, it is important to know if exercise training can reverse the arterial stiffening and produce improvements in the CVD risk profile of MetS individuals prior to overt T2DM or CVD. Understanding the

mechanisms mediating favorable changes in arterial function prior to the progression to T2DM are important. It is possible that the changes in the arterial expression, architecture, and/or bioactivity of structural proteins that play a major role with CVD, are either reversed or ameliorated through exercise. Favorable effects of exercise on improving the balance with pro-and anti-inflammatory and oxidative stress markers could improve nitric oxide bioavailability, and alter matrix metalloproteinases (MMPs), and/or tissue inhibitors of MMP (TIMPs) activity. MMP are regulators of tissue remodelling and its activity is greatly affected by mechanical stretch. Chronic exercise training, which acts as a mechanical stressor to the arteries, could exert its effects by altering MMP/TIMP activity directly and/or indirectly via the anti-inflammatory and oxidative stress responses of exercise.

We examined whether aerobic exercise training can reverse arterial stiffness and improve arterial mechanics including central pressures in individuals with MetS without overt CVD or T2DM. Further, we examined the potential mechanisms (inflammation, and MMP/TIMP activity) through which aerobic exercise may improve arterial health. We hypothesized that exercise training in MetS would reduce central arterial stiffness compared to the non-trained MetS control group.

Methods

Study Population

21 healthy controls (41 ± 2 years; 71% female) and 22 MetS (45 ± 2 years; 60% female) subjects participated in the study. The healthy controls were free from CVD, as determined by a detailed history, physical examination, and a normal resting and exercise electrocardiogram. MetS subjects were free from overt CVD and diabetes. Further, none of the participants were current or former smokers or were being treated for peripheral artery disease. MetS was defined according to the updated National Cholesterol Education Program: Adult Treatment Panel III [13] comprised of 3 out of the following 5 components: 1) obesity (waist men >102 cm, women >88 cm); 2) low HDL cholesterol (men <40 mg/dL; women <50 mg/dL); 3) hypertriglyceridemia (≥ 150 mg/dL); 4) elevated glucose (≥ 100 mg/dL & <126 mg/dL), and; 5) elevated BP (130/85 mmHg or use of hypertensive medications).

Exclusion criteria included T2DM (HbA1c ≥ 6.5 % or use of diabetic medications), pulmonary disease, angina, atrial fibrillation, aortic stenosis, anemia, myocardial infarction, stroke, or coronary revascularization as assessed by a detailed medical history physical examination, and a resting and exercise electrocardiogram. Subjects who participated in regular exercise, defined as >30 minutes, 3 times/week were excluded. All subjects provided written informed consent to participate that was submitted to and approved by WVU Institutional Review Board.

Study Design.

Assessments were performed between 7:00-10:00 AM, in a quiet, temperature-controlled room, after a 12 hour fast, and abstinence from alcohol, caffeine, and vitamins. Cardiovascular medications were withheld 24 hours prior to assessments. Upon completion of the anthropometric assessments and after a minimum 15 minutes of quiet supine rest, subjects underwent supine measures of arterial structure/function followed by a blood draw.

Body Anthropometry

Height, and weight, along with waist and hips circumference were measured using standard laboratory procedures. Fat distribution was assessed by measuring the waist circumference at the site of the smallest circumference between the rib cage and the ileac crest with the subjects in standing position. Hip circumference was measured at the site of the largest circumference between waist and thighs. Body composition was calculated from body volume of the BodPod® (Life measurement, Concord, CA, USA). Subjects wore tightly fitting bathing suits and a swim cap during the volume-measurements in the BodPod®. Body mass index (BMI) was calculated as weight (kg)/height (m)².

Arterial Geometry

Ultrasound (GE Vivid i) 2-D images of the right common carotid artery were obtained 1-2 cm proximal to the carotid bifurcation to measure maximal

lumen diameter, and intima-medial thickness (cIMT). Cross-sectional area of the carotid artery was calculated as $[(\text{maximal lumen diameter}/2)^2 \times \pi] - (\text{maximal lumen diameter}/2 - \text{cIMT})^2 \times \pi$. The wall to lumen ratio of the right common carotid artery (CCA W/L) was calculated as $2 \times \text{cIMT}/\text{lumen diameter}$ in diastole [2].

Arterial Function

Brachial systolic (SBP) and diastolic (DBP) blood pressure was measured with an automated, oscillometric, sphygmomanometer (Critikon Dinamap Compact BP monitor, GE Medical, Tampa, Fla , USA) and pulse pressure (PP) was calculated from SBP-DBP. Pulse wave analysis was performed noninvasively on the radial artery (SphygmoCor system, AtCor Medical, Sydney, Australia). All measurements were made in triplicate, and the mean values used for subsequent analysis. The SphygmoCor system synthesizes a central (ascending aortic) pressure waveform from the radial pressure waveform that does not differ from that of an intra-arterially recorded wave [14] using a validated generalized transfer function [15] that has good reproducibility under major hemodynamic changes [16]. These waveforms were calibrated against brachial mean arterial and diastolic pressure to estimate aortic pressures.

The characteristics of the aortic pulse wave (Figure 1) were determined as follows using established guidelines [17]. Forward wave pressure was defined as the difference between pressure at the waveform foot and the pressure at the inflection point and used as an index of peak left ventricular ejection velocity (P1,

Figure 1). Left ventricular (LV) systolic ejection duration was taken as the time from the foot of the pressure wave upstroke to the incisura of the aortic notch. Augmented pressure (AP), a measure of the contribution of wave reflections to SBP, was defined as the difference between aortic SBP and the pressure at the forward wave peak. Augmentation index (AGI) provides a measure of the contribution of wave reflection pressure (i.e. AP) to SBP relative to total PP. AGI was calculated as the ratio of amplitude of the pressure wave above its systolic shoulder (i.e. the difference between the early and late systolic peaks of the arterial waveform), to the total PP expressed as a percentage $(P_2 - P_1 / PP * 100)$ [14]. Since AGI varies with heart rate, AGI is commonly adjusted to a 'standard heart rate' of 75 beats per minute (AGI@75). Travel time of the forward pressure wave from the aorta to the peripheral reflection site and back (T_r) was determined from the time from the initial upstroke of the pressure wave to the foot of the reflection wave and used as a proxy of aortic pulse wave velocity. Systolic-to-diastolic pressure shifts were assessed by the systolic (red shaded area, Figure 1) and diastolic (blue shaded area, Figure 1) pressure-time integrals. The Buckberg sub-endocardial viability ratio (SEVR) index, which correlates with LV sub-endocardial:sub-epicardial flow ratio (an apparent marker of sub-endocardial ischemia) [18], was calculated as the percentage ratio of the diastolic (diastolic time index, DTI):systolic (tension time index, TTI) pressure-time integral.

Carotid to femoral pulse wave velocity (cfPWV; central arterial stiffness) and carotid to radial pulse wave velocity (crPWV: peripheral arterial stiffness)

were measured by applanation tonometry (AtCor Medical, Sydney, Australia). ECG-gated waveforms were sequentially recorded. Aortic distance (D) was calculated as the difference in the distances from the carotid to the suprasternal notch and from the suprasternal notch to the femoral artery or radial artery. Time delay was calculated using a foot-of-the-wave method.

Using B-mode ultrasound (GE Vivid i) the right CCA strain was calculated as $(\Delta D_c)/D \times 100$, where ΔD_c is the difference between systolic and diastolic carotid diameter, D is the diastolic diameter. CCA circumferential wall stress = Mean BP x CCA W/L. Peak CCA circumferential wall tension was calculated as aortic SBP x $(D_c \text{ in systole}/2)$

Blood Analysis

Venous blood sampling was obtained in the morning after a 12-hour overnight fast. Post training blood samples were collected at least 48 hours after the last exercise session. Serum and plasma was obtained from blood samples collected in either serum separation tubes with polymer gel/silica activator (BD Vacutainers®) or plasma separation tubes (BD Vacutainers®). Using standard procedures total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and insulin were processed at West Virginia University Hospital's central laboratory in Morgantown, West Virginia. Homeostasis model assessment of insulin resistance (HOMA-IR) was estimated with the following formula: insulin resistance = fasting plasma insulin (in microunits per mL $\mu\text{U/mL}$) $\times$ fasting plasma glucose (FPG, in millimoles per litre)/22.5. HOMA β -cell function

was also assessed from 360* fasting insulin/(fasting glucose-63). Plasma MMP (1, 2, 7, 9, and 10) and TIMP (1 and 2) activity, serum adhesion molecules (Serum Endothelial Leukocyte Adhesion Molecule-1, soluble cell adhesion molecules-1, vascular cell adhesion molecule-1, and tissue plasminogen activator inhibitor-1), and serum inflammatory markers (IL-6, IL-8, and TNF α) were run on a Luminex® 100™ Bioanalyzer (Luminex Corp., Austin, TX, USA) according to the kit manufacturer's (Milliplex ELISA kits, Millipore Corporation, MA, USA) instructions. The reader is referred to the online supplement for further details on blood processing.

CVD Risk

The Framingham Risk Score (FRS) was calculated based on sex, age, brachial SBP, treatment for hypertension, smoking and diabetes status, HDL, and total cholesterol to establish an individual's general 10 year CVD risk, along with their estimated Framingham vascular age [19]. Further, we also estimated vascular age based on published sex-specific algorithms for cfPWV (PWVage) [20].

Intervention

Individuals were assigned into either an 8-week aerobic exercise intervention group (ExT) or an 8 week non-exercise control (Non-ExT) group. Initially, 11 controls were assigned to the Non-ExT group however, one dropped out for personal reasons. Data were only analyzed on subjects who completed

the study. The Non-ExT exercise training groups (Controls: n=10, 80% female; MetS: n=11; 55% female) were instructed to maintain their normal lifestyle activities. The ExT groups (Controls: n=11, 64% female; MetS: n=11; 73% female) performed 8 weeks of supervised aerobic exercise, 3 days/week for 60 min/day at a fixed exercise intensity performed in the Human Performance Lab at West Virginia University. The intensity of prescribed exercise was based on individual results of maximal upright bicycle cardiopulmonary exercise tests with the resistance to peddling increasing by 25 watts every 3 minutes until subjects reached exhaustion. Subjects were required to pedal at 50 revolutions/minute. We deployed a ramp exercise protocol, whereby the exercise intensity started at 60% of heart rate reserve (heart rate range determined during exercise stress test) and increased every 2 weeks by 10%; from weeks 6-8 heart rate reserve was set at 85%. Adherence to the exercise prescription was documented through the use of Portable heart rate monitors (E600, Polar Electro OY, Oulu, Finland) and physical activity logs. Approved modalities included treadmills, elliptical machines, and cycle ergometers. The participants were instructed to maintain current eating behaviors for the duration of the 8-week intervention. All post-training measurements were performed at least 48 hours after the last exercise session to avoid the immediate effects of a single bout of exercise. In addition, measurements before and after intervention periods were obtained at the same time of day for each subject.

Statistical Analysis

Our sample size calculation for our primary outcome measures (cfPWV) assumed a power of 90% and an alpha error probability of 0.05. We found that we required a sample size of at least 11 subjects to detect a clinically significant effect of exercise training on arterial stiffness such as a difference in cfPWV of 1 ± 1 (SD) m/s. Normality was evaluated by the Kolmogorov-Smirnov test. Categorical variables were compared by the chi-square test. Continuous variables were log transformed as necessary and pre-exercise variables were compared between groups with a One-way ANOVA with a Tukey's post hoc test. To evaluate the effects of exercise training paired *t*-tests and two-way repeated-measures ANOVA were used. All analyses were performed with the statistical package SPSS version 21 (SPSS, Chicago, IL). Values shown in the tables represent means $\pm$ SEM unless otherwise stated. *P* <0.05 was defined as significant.

Results

Age, anthropometric and metabolic characteristics of the healthy controls and MetS groups are shown in Table 1. MetS and controls groups did not differ by age or sex; however, by study design the MetS groups had significantly higher anthropometric and metabolic characteristics. Additionally, pre-exercise peak VO_2 was lower ($p < 0.05$) in MetS NonT (18 ± 2 ml/min/kg) and MetS ExT (16 ± 1 ml/min/kg) groups vs. healthy NonT (25 ± 2 ml/min/kg) and healthy ExT (24 ± 2 ml/min/kg) controls. However, within each group (i.e., MetS Non-ExT vs. ExT, and controls Non-ExT vs. ExT) individuals were well matched with no significant differences in age, baseline metabolic and arterial parameters.

Arterial Parameters

It is well known that MetS patients have higher central and brachial pressures, an increased arterial stiffness, and arterial remodeling compared to healthy controls. Similar differences were evident in our population (Table 2). In healthy controls, 8 weeks of exercise training significantly reduced cfPWV and AGI, and there was a significant time (Pre vs. Post) by group (Non-ExT vs. ExT) group interaction for cfPWV. However, no other significant differences in arterial structure/function were found pre-and post-exercise training in healthy controls. Importantly, 8 weeks of exercise training in MetS patients reduced brachial and central SBP, although only a significant reduction was identified in the central SBP ($p < 0.05$). Further, AP was significantly reduced (20%, $p < 0.05$) in the MetS-ExT group. Although AGI was not affected by exercise training, a reduction (21%,

$p=0.06$) in AGI@75HR was identified. Indeed, resting HR was reduced (6%, $p<0.05$) in MetS-ExT group. Due to the reduction in central SBP the systolic region of the pressure wave was reduced (9%, $p<0.05$) along with an improvement in SEVR (8%, $p<0.05$) in the MetS-ExT group, and there was a significant time (Pre vs. Post) by group (Non-ExT vs. ExT) interaction for SEVR. Importantly, aerobic training lowered cfPWV in MetS individuals by 9% ($p<0.05$), which remained significant after adjusting for differences in MAP. Further, there was a significant time (Pre vs. Post) by group (Non-ExT vs. ExT) group interaction for cfPWV. In contrast, 8 weeks of exercise training had no effect on improving crPWV, cIMT, carotid cross-sectional area, carotid lumen to wall ratio, carotid wall stress or tension in MetS-ExT.

In the Non-ExT controls and MetS, no differences in arterial structure and function were found 8 weeks post-intervention compared to baseline values (Table 2).

Blood Biomarkers

We also examined the effects of exercise training on improving metabolic markers, inflammation, and vascular proteins circulating in the blood. No differences in metabolic markers, adhesion or the inflammation markers were evident in either the healthy controls or MetS group after the intervention (Table 3). However, a reduction in MMP-1 ($p<0.05$), and to a lesser extent MMP-7 ($p=0.06$), was evident after exercise training in the MetS group, but not in the

healthy control group. A significant time (pre and post intervention) by group (MetS NonT vs. MetS ExT) interaction was identified for MMP-7.

Aerobic Capacity and CVD risk

Aerobic capacity was increased ($p<0.05$) in MetS (16.6 ± 1.0 to 19.9 ± 1.0) and healthy controls (23.8 ± 1.6 to 26.3 ± 1.6) after exercise training, whereas no changes were found in MetS (18.5 ± 1.6 vs. 18.2 ± 1.2) and healthy controls (25.6 ± 2.8 vs. 25.8 ± 2.5) who remained inactive. As expected the FRS and the vascular age calculated from the Framingham Risk algorithm were higher ($p<0.05$) in both MetS groups compared to healthy controls (Table 1). Exercise training did not affect the FRS in MetS ($8\pm4\%$ vs. 6 ± 3) or healthy controls ($2\pm1\%$ vs. 2 ± 1). However, in MetS exercise training significantly reduced vascular age calculated from the Framingham Risk algorithm (from 47 ± 8 to 44 ± 7 years, $p=0.02$), and from cfPWV (from 55 ± 5 to 50 ± 4 years, $p=0.04$). Adding PWVage to the FRS tended to reduce the calculated CVD risk (10 ± 5 vs. $6\pm3\%$), but statistical significance was not reached ($p=0.15$). In contrast, neither vascular age calculated from the FRS (34 ± 4 vs. 33 ± 4 years, $p=0.6$) or from cfPWV (40 ± 3 vs. 37 ± 4 years, $p=0.1$) was altered in the healthy controls ExT group. Similarly, adding PWV to the FRS did not alter their calculated CVD risk ($3\pm1\%$ vs. $2\pm1\%$).

Relationship between Aerobic Capacity, and Arterial Health.

An inverse relationship ($p<0.05$) was identified between VO_{2peak} , and cfPWV (Figure 2A), cIMT (Figure 2B), AP (Figure 2C), and both central and

brachial SBP (Figure 2D). We also calculated the change (post-pre values) in cfPWV, cIMT, AP, AGI, AGI@HR75, and both brachial and central SBP, and compared that to the change in VO_{2peak} . A significant and inverse relationship was found only between VO_{2peak} and cfPWV (Figure 3).

Discussion

Our laboratory [2] and others [3] have shown that the MetS is associated with arterial dysfunction, reflected by an increase in arterial stiffness, cIMT, carotid cross-sectional area, carotid wall stress and tension. Part of this arterial dysfunction reflects the sedentary lifestyle of individuals with MetS, and physical inactivity is independently associated with cardiovascular and all-cause mortality [21]. The present study is novel in that we examined whether aerobic exercise training would be an effective non-pharmacological therapy to lower arterial stiffness and improve other markers of arterial health in MetS patients who had yet to transition to more adverse stages of the cardiometabolic disease such as the co-occurrence of T2DM, or the presence of overt CVD (angina, heart attack, stroke etc). Of particular importance, cfPWV, which is increased in MetS (even without overt CVD) compared to age-matched healthy controls, is significantly reduced after 8 weeks of aerobic exercise in MetS by almost 1 m/s. Given the clinical significance of increased arterial stiffness, this reduction in cfPWV after a short period of exercise training likely reflects a reduction in CV risk.

In the present study, the reduced cfPWV in MetS after exercise training occurred in the absence of significant improvements in weight, BMI, and metabolic profile, suggests the effects of aerobic training on the arterial stiffness may be independent of these other well-established benefits of exercise. The reduction in cfPWV coincided with a reduction in central SBP, AP, AGI, and an improvement in sub-endocardial variability. The average reduction in SBP with aerobic exercise training is 3.9 mmHg and is independent of body weight and

race [22]. We found that 8 weeks of exercise training reduced brachial and central SBP between 5-6 mmHg in MetS. As central SBP predicts cardiovascular outcomes more closely than brachial BP [23], the reduction in cSBP is clinically relevant. Given that arterial stiffness and augmentation pressure play key roles in determining central SBP, the reduction in central SBP can be attributed in part to a reduction in arterial stiffness, and a reduction AP. Central AP represents the amount of pressure added to the systolic pressure peak based on the reflected wave. A decrease in arterial stiffness can result in a corresponding decrease in the transmission velocity of the forward and reflected waves causing the reflected wave to arrive later in the central aorta and augment pressure in late systole or early diastole. Thus, once AGI (an indirect measure of systemic arterial stiffness), was adjusted to a standard HR (AGI@HR75) a reduction in the reflected pressure wave was evident. Adjusting for HR in our study was important given that exercise training in MetS was associated with a 4 bpm reduction in HR. Effectively, a decrease in HR will extend the duration of the cardiac cycle, shifting the reflected wave into systole and increasing AP. In addition to the effects of HR, wave reflection is also dependent on arterial stiffness [24], which decreased to a greater extent than HR in exercised trained MetS. The improvements in central pressure dynamics corresponded to an increase in SEVR, an indirect marker of subendocardial perfusion [18], which may suggest an improvements in cardiac function.

Aerobic exercise training did not alter cIMT, carotid cross-sectional area, or wall to lumen ratios, or carotid dynamics (CCS strain, wall stress or wall

tension) in MetS. This lack of change in cIMT is similar to that reported elsewhere in middle-aged and older healthy men [25, 26] suggesting that regular exercise training typically does not alter the large conduit vessels.

Our data might suggest that exercise training exerted greater benefits to MetS than healthy controls as evident by the larger exercise-induced decreases in cSBP, AP, TTI and increase in SEVR in MetS compared to healthy controls. Likewise, MetS patients also exhibited a tendency to have greater reduction in cfPWV (0.7 vs. 0.3 m/s) and VO_{2peak} (3.3 vs. 2.5 ml/min/kg) than controls. However it must be emphasized no significant ANOVA interaction effects were observed between MetS and healthy controls with these pre-post training responses, and that our study was also not originally powered to test this question. Nevertheless, it is tempting to speculate that potential for greater improvement in these parameters for MetS patients may be due to their elevated baseline compared to controls and therefore could provide a greater range for the exercise stimulus to act on. This is an important question that will require additional research/subjects before a clear understanding is gained.

Recent evidence suggests that any initial benefit with exercise training on arterial stiffness in persons with established MetS and T2DM is attenuated over the long term [11, 27]. Whereby, at 3 months of aerobic exercise training a 14-23% reduction in cfPWV has been reported [11, 28], yet after 6 months of exercise training the reduction in cfPWV was not maintained [11]. These data suggest that the prescription of aerobic exercise on improving arterial stiffness may be more beneficial prior to the occurrence of T2DM in those with MetS, than

starting once the patient is already at a high cardiometabolic risk. To what extent this initial reduction in arterial stiffness through short-term exercise training in MetS without T2DM is maintained for an extended period of time, and whether exercise training helps to delay or prevent the transition from MetS to T2DM warrants further investigation.

Potential mechanism behind improved arterial stiffness

The stiffness of the arterial system is dependent on the dynamic (autonomic tone, smooth muscle cell contractility, endothelial function) and material properties (elastin content and integrity, collagen content, fibrosis, elastin and collagen cross-linking, calcification) of the artery [29]. Sympathetic activation of the vascular smooth muscle cells causes vasoconstriction, decreasing lumen diameter, and increasing arterial stiffness. Although we did not measure sympathetic activity, exercise training has been shown to improve sympathetic -and parasympathetic nervous activities in obese individuals [30, 31]. Thus, it is possible that the improvement in arterial stiffness in the current study may be in part due to improved autonomic function.

Arterial stiffness is also in part modulated by endothelial dysfunction. Exercise training in both obese/MetS patients has been shown to improve endothelial function [32, 33], which may improve arterial function via intermittent increases in shear stress, which stimulates nitric oxide bioavailability, and up-regulating endothelial NO synthase protein expression and phosphorylation [34]. Changes in distending pressure affect arterial stiffness, where at higher

pressures the force on the arterial wall is transferred to the stiffer collagen limiting distention. In our study, MAP did not change, and the decrease in cfPWV in the training groups remained significant after adjusting for MAP. Thus, it is unlikely that a reduction in distending pressure contributed to the improved arterial compliance.

In terms of the material properties that effect arterial stiffness, the main contributors to increased arterial stiffness are increased collagen concentration, non-enzymatic glycation resulting in the formation of collagen crosslink, and vascular smooth muscle hypertrophy [29]. Hyperglycemic conditions, and oxidative stress, accelerate the generation of advanced glycation end-products precursors contributing to variable pathologies such as T2DM, MetS and obesity. In our study, we did not measure the amount of advanced glycation end-product deposition; however we did measure plasma markers of tissue remodelling (MMP and TIMP) and found that exercise training lowered circulating MMP-1 and 7. Interestingly, both MMP-1 and 7 were positively associated with cfPWV. More evidence is required to identify to what extent MMPs play a role in arterial remodeling through exercise training.

Chronic low grade inflammation, specifically IL-6, IL-8, and TNF α have been postulated to be involved in the pathogenesis of MetS [35] and arterial stiffness [36], and in the present these markers tended to be elevated in MetS vs. controls. However, we found that exercise training did not alter inflammatory markers in MetS. In contrast, improvements in TNF α , and IL-18 have been found after 12 weeks and 1 year of exercise training in MetS with or without T2DM [37,

38]. Thus, the lack of change in inflammatory state in our MetS group after exercise training maybe due to the shorter duration of exercise we used.

Clinical implications

A strong relationship exists between arterial stiffness and CVD mortality [4] and in our study the decrease in cfPWV by almost 1 m/s may reflect a lowering of CVD events and mortality by approximately 14% [4]. The reduction in cfPWV coincided with a reduction in central SBP, which is important given that a reduction SBP by 2 mmHg is associated with a 10% and 7% decrease in mortality from cerebral vascular and CVD events [39]. Arterial stiffness contributes substantially to cardiac workload and energetics, and is inversely correlated with VO_{2peak} [40, 41]. We have confirmed this relationship and shown that the change in VO_{2peak} and cfPWV with exercise training are inversely related. These data suggest that individuals with more compliant arteries may have more optimal coupling between the heart and arterial system allowing for greater peak CV performance. Indeed, arterial-ventricular coupling is a critical determinant of net CV performance [42]. It has been reported that MetS with a high aerobic fitness have a lower risk of mortality than unfit MetS [43], which may in part be explained by the lower degree of arterial stiffening [41].

Increasing arterial stiffness is regarded as a measure of premature vascular aging [44]. According to normative reference values of arterial stiffness [45], the baseline biologic vascular age of our MetS group may be estimated to be 6 years older than it would be in healthy individuals of a similar age. The

reduction in cfPWV observed in the MetS ExT group may translate into a reversal of age-related arterial stiffening of 5 years [46]. Adding vascular age calculated from cfPWV to the FRS lowered the calculated CVD risk by 4%.

Future directions

The mechanisms responsible for improving arterial function and aerobic capacity in MetS patients after exercise training likely reflect multiple CV adaptations some of which we have pointed out. However, the specific roles of the sympathetic nervous system, the structure and function of resistance vessels, and oxidative stress require further examination in both human and animal models. Given the lack of change in metabolic profile of our MetS population it would be important to identify whether exercise training of longer durations (6 months plus), or exercise training plus diet control would exert greater benefits on both arterial function and metabolic profile of MetS patients. It would also be important to identify whether the beneficial effects of short term exercise training on lowering arterial stiffness is maintained over a longer period of time or indeed is it lost as evident in T2DM patients. Further, it will also be important to conduct longitudinal studies to identify whether persistent exercise training in MetS can delay or prevent the transition to MetS with overt CVD/diabetes.”

Limitations

There are several limitations. There may be sex differences in the effects of exercise training on arterial stiffness that could not be detected given the small

number of male vs. female subjects. Second, more direct measures of autonomic function should be used to assess whether changes in sympathetic activity contributed to a reduction in cfPWV. Another limitation is the circulating levels of MMP's do not necessary reflect what is going on at the arterial sites. However, because the changes on the cellular level are reflected in body fluids, determination of MMPs in blood have been recommended as noninvasive tools in the diagnosis and monitoring of several diseases [47]. Although we reviewed each participant medical history and performed a physical examination we cannot completely rule out that participants may have had some degree of peripheral artery disease. Despite this exercise training in MetS (with or without peripheral artery disease) significantly improved arterial health. Four of our MetS patients had a BMI >40 kg/m² indicating they were severely obese. However, being severely obese did not limit their ability to improve arterial function, as we repeated the statistical analyses excluding these individuals and found that the main story of the paper did not change. Lastly, a potential limitation is the measurement of plasma lipids obtained from lithium heparin coated collection tubes, given that heparin precipitates some lipoproteins. This may explain, in part, the lack of change in blood lipids post-exercise training the short duration of exercise training.

In conclusion, the present study indicates that aerobic exercise training is an effective approach to reduce arterial stiffening and improve CVD risk profiles in individuals with the MetS (without T2DM and overt CVD). Whether long-term

aerobic exercise training maintains the beneficial effects on arterial stiffness in MetS and delays the transition to T2DM requires further study.

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Disclosures

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Reference List

1. Malik, S., et al., *Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults*. *Circulation*, 2004. 110(10): p. 1245-1250.
2. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. *Experimental Physiology*, 2013. 99(1): p. 149-163.
3. Scuteri, A., et al., *The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study*. *European Heart Journal*, 2010. 31(5): p. 602-613.
4. Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis, *Prediction of Cardiovascular Events and All-Cause Mortality With Arterial Stiffness: A Systematic Review and Meta-Analysis*. *Journal of the American College of Cardiology*, 2010. 55(13): p. 1318-1327.
5. Lorenz, M.W., et al., *Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness*. *Circulation*, 2007. 115(4): p. 459-467.
6. Tanaka, H., et al., *Aging, habitual exercise, and dynamic arterial compliance*. *Circulation*, 2000. 102(11): p. 1270-5.
7. Currie, K.D., S.G. Thomas, and J.M. Goodman, *Effects of short-term endurance exercise training on vascular function in young males*. *Eur J Appl Physiol*, 2009. 107(2): p. 211-8.

8. Ferrier, K.E., et al., *Aerobic exercise training does not modify large-artery compliance in isolated systolic hypertension*. Hypertension, 2001. 38(2): p. 222-6.
9. Seals, D.R., et al., *Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness*. J Am Coll Cardiol, 2001. 38(2): p. 506-13.
10. Miyaki, A., et al., *Effect of Habitual Aerobic Exercise on Body Weight and Arterial Function in Overweight and Obese Men*. The American Journal of Cardiology, 2009. 104(6): p. 823-828.
11. Madden, K.M., et al., *Aerobic training-induced improvements in arterial stiffness are not sustained in older adults with multiple cardiovascular risk factors*. J Hum Hypertens, 2013. 27(5): p. 335-9.
12. da Silva, C.A., et al., *High-intensity aerobic training improves endothelium-dependent vasodilation in patients with metabolic syndrome and type 2 diabetes mellitus*. Diabetes Res Clin Pract, 2012. 95(2): p. 237-45.
13. Huang, P., *A Comprehensive Definition for Metabolic Syndrome*. Disease Models & Mechanisms, 2009. 2(2): p. 231-237.
14. Chen, C.-H., et al., *Validation of Carotid Artery Tonometry as a Means of Estimating Augmentation Index of Ascending Aortic Pressure*. Hypertension, 1996. 27(2): p. 168-175.
15. Chen, C.-H., et al., *Estimation of Central Aortic Pressure Waveform by Mathematical Transformation of Radial Tonometry Pressure: Validation of Generalized Transfer Function*. Circulation, 1997. 95(7): p. 1827-1836.

16. Sharman, J.E., et al., *Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise*. Hypertension, 2006. 47(6): p. 1203-8.
17. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications*. Eur Heart J, 2006. 27(21): p. 2588-605.
18. BUCKBERG, G.D., et al., *Experimental Subendocardial Ischemia in Dogs with Normal Coronary Arteries*. Circulation Research, 1972. 30(1): p. 67-81.
19. D'Agostino, R.B., Sr, et al., *General Cardiovascular Risk Profile for Use in Primary Care: The Framingham Heart Study*. Circulation, 2008. 117(6): p. 743-753.
20. McEniery, C.M., et al., *Normal Vascular Aging: Differential Effects on Wave Reflection and Aortic Pulse Wave Velocity: The Anglo-Cardiff Collaborative Trial (ACCT)*. J Am Coll Cardiol, 2005. 46(9): p. 1753-1760.
21. Blair, S.N., et al., *Changes in physical fitness and all-cause mortality: A prospective study of healthy and unhealthy men*. JAMA, 1995. 273(14): p. 1093-1098.
22. Whelton, S.P., et al., *Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials*. Ann Intern Med, 2002. 136(7): p. 493-503.

23. Wang, K.L., et al., *Central or peripheral systolic or pulse pressure: which best relates to target organs and future mortality?* J Hypertens, 2009. 27(3): p. 461-7.
24. Papaioannou, T.G., et al., *The Effect of Heart Rate on Wave Reflections May Be Determined by the Level of Aortic Stiffness: Clinical and Technical Implications.* American Journal of Hypertension, 2008. 21(3): p. 334-340.
25. Tanaka, H., et al., *Regular aerobic exercise and the age-related increase in carotid artery intima-media thickness in healthy men.* Journal of Applied Physiology, 2002. 92(4): p. 1458-1464.
26. Thijssen, D.H.J., et al., *Vascular adaptations to 8-week cycling training in older men.* Acta Physiologica, 2007. 190(3): p. 221-228.
27. Dobrosielski, D., et al., *Effect of Exercise on Blood Pressure in Type 2 Diabetes: A Randomized Controlled Trial.* Journal of General Internal Medicine, 2012. 27(11): p. 1453-1459.
28. Madden, K.M., et al., *Short-Term Aerobic Exercise Reduces Arterial Stiffness in Older Adults With Type 2 Diabetes, Hypertension, and Hypercholesterolemia.* Diabetes Care, 2009. 32(8): p. 1531-1535.
29. Ziemann, S.J., V. Melenovsky, and D.A. Kass, *Mechanisms, pathophysiology, and therapy of arterial stiffness.* Arterioscler Thromb Vasc Biol, 2005. 25(5): p. 932-43.
30. Figueroa, A., et al., *Endurance training improves post-exercise cardiac autonomic modulation in obese women with and without type 2 diabetes.* Eur J Appl Physiol, 2007. 100(4): p. 437-44.

31. Amano, M., et al., *Exercise training and autonomic nervous system activity in obese individuals*. Med Sci Sports Exerc, 2001. 33(8): p. 1287-91.
32. Tjonna, A.E., et al., *Aerobic Interval Training Versus Continuous Moderate Exercise as a Treatment for the Metabolic Syndrome: A Pilot Study*. Circulation, 2008. 118(4): p. 346-354.
33. Mestek, M.L., et al., *Regular aerobic exercise, without weight loss, improves endothelium-dependent vasodilation in overweight and obese adults*. Obesity (Silver Spring), 2010. 18(8): p. 1667-9.
34. Green, D.J., et al., *Effect of exercise training on endothelium-derived nitric oxide function in humans*. The Journal of Physiology, 2004. 561(1): p. 1-25.
35. Ahonen, T.M., et al., *The association of adiponectin and low-grade inflammation with the course of metabolic syndrome*. Nutrition, Metabolism and Cardiovascular Diseases, 2012. 22(3): p. 285-291.
36. Mahmud, A. and J. Feely, *Arterial stiffness is related to systemic inflammation in essential hypertension*. Hypertension, 2005. 46(5): p. 1118-1122.
37. Balducci, S., et al., *Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss*. Nutrition, Metabolism and Cardiovascular Diseases, 2010. 20(8): p. 608-617.

38. Stensvold, D., S.A. Slordahl, and U. Wisloff, *Effect of exercise training on inflammation status among people with metabolic syndrome*. Metab Syndr Relat Disord, 2012. 10(4): p. 267-72.
39. Lewington, S., et al., *Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies*. Lancet, 2002. 360(9349): p. 1903-13.
40. Vaitkevicius, P.V., et al., *Effects of age and aerobic capacity on arterial stiffness in healthy adults*. Circulation, 1993. 88(4 Pt 1): p. 1456-62.
41. Jae, S.Y., et al., *Association between cardiorespiratory fitness and arterial stiffness in men with the metabolic syndrome*. Diabetes Res Clin Pract, 2010. 90(3): p. 326-32.
42. Chantler, P.D., E.G. Lakatta, and S.S. Najjar, *Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise*. Journal of Applied Physiology, 2008. 105(4): p. 1342-1351.
43. Katzmarzyk, P.T., T.S. Church, and S.N. Blair, *Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men*. Arch Intern Med, 2004. 164(10): p. 1092-7.
44. Nilsson, P.M., P. Boutouyrie, and S. Laurent, *Vascular aging: A tale of EVA and ADAM in cardiovascular risk assessment and prevention*. Hypertension, 2009. 54(1): p. 3-10.
45. Collaboration, T.R.V.f.A.S., *Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing*

- normal and reference values*'. European Heart Journal, 2010. 31(19): p. 2338-2350.
46. Avolio, A.P., et al., *Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community*. Circulation, 1983. 68(1): p. 50-8.
47. Mannello, F., et al., *Differences in both matrix metalloproteinase 9 concentration and zymographic profile between plasma and serum with clot activators are due to the presence of amorphous silica or silicate salts in blood collection devices*. Analytical Biochemistry, 2008. 374(1): p. 56-63.

Table 1: Clinical Characteristics of the subject cohorts

	Controls NonT			Controls ExT			MetS NonT			MetS ExT		
Age, years	40	±	4	41	±	4	44	±	3	46	±	4
Sex, female %	80%			64%			64%			73%		
Height, cm	165	±	3	168	±	2	171	±	3	168	±	3
Weight, kg	68	±	4	69	±	4	102	±	7*^	106	±	5*
Body Fat, %	25	±	4	24	±	2	36	±	2*^	45	±	2*
BSA, m ²	1.75	±	0.06	1.78	±	0.06	2.13	±	0.08*^	2.13	±	0.06*
BMI, kg/m ²	25	±	1	24	±	1	34	±	2*^	38	±	2*
Waist circumference, cm	83	±	3	86	±	6	107	±	3*^	124	±	9*
Total Cholesterol, mg/dL	191	±	11	177	±	12	189	±	12	191	±	12
Triglycerides, mg/dL	93	±	14	81	±	14	161	±	23*^	115	±	16
HDL, mg/dL	56	±	5	57	±	4	40	±	4*^	50	±	5
Glucose, mg/dL	93	±	3	92	±	3	97	±	1	98	±	2
HbA1c, %	4.9	±	0.3	5.4	±	0.1	5.7	±	0.1*	5.6	±	0.1*
Insulin, µIU/mL	5	±	1	5	±	1	11	±	2*^	10	±	1*^
Beta Cell function	62	±	10	79	±	37	111	±	20*^	105	±	16*^
HOMA IR	1.3	±	0.2	1.0	±	0.3	2.7	±	0.5*^	2.4	±	0.3*^
Framingham Risk Score, %	2	±	1	2	±	1	8	±	2*^	8	±	4*^
Framingham Vascular Age, years	36	±	3	34	±	4	54	±	4*^	47	±	7*^
Hypertensive %	0			0			73			82		
Hypertension category												
Normal, %	40			64			18			18		
Pre-hypertension, %	10			18			0			0		
Stage 1, %	0			0			82			73		
Stage 2, %	0			0			0			1		
Diabetes Mellitus %	0			0			0			0		
Smokers, %	0			0			0			0		
Medications												
Diuretics, %	0			0			10			18		
B-blockers, %	0			0			0			18		
ACEI/ARB, %	0			0			36			18		
CA ²⁺ blockers, %	0			0			0			0		
Statins, %	0			0			0			18		

Values are mean ± 1 SEM; BSA: body surface area; BMI: body mass index; HDL: high density lipoprotein; HbA1c: hemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance.

*p<0.05 compared to healthy control NonT group; ^ p<0.05 compared to healthy control ExT group;

Table 2: Differences in arterial parameters pre and post intervention in MetS and controls

	Controls NonT (n=10)		Controls ExT (n=11)		MetS NonT (n=11)		MetS ExT (n=11)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
bSBP (mmHg)	117±3	115±4	111±3	110±3	128±4 [^]	126±4	125±5 [^]	120±4
bDBP (mmHg)	74±2	75±3	73±2	71±2	84±3 [*]	81±2	80±2 [*]	78±2
bPP (mmHg)	42±3	41±2	38±2	39±2	43±3	45±3	46±5	42±3
bMAP (mmHg)	91±3	88±3	87±2	85±2	100±3 [*]	97±2	96±2 ^{*^}	93±3
cSBP (mmHg)	107±3	104±4	103±3	101±2	117±4 [^]	114±3	116±5 [^]	110±4†
cPP (mmHg)	31±2	30±2	29±2	28±2	32±2 ^{*^}	33±2	36±5	32±3
P1 H (mmHg)	24±2	23±1	22±2	23±1	25±2	26±2	27±3	25±2
AP (mmHg)	7.2±1.7	6.5±1.8	6.6±1.8	5.6±1.6	6.9±1.4	6.7±1.3	8.9±1.4	7.1±1.4†
AGI (%)	22±5	20±5	21±5	18±5†	21±4	20±3	24±2	23±4
AGI@75HR (%)	18±4	16±5	14±5	11±4	17±4	16±3	19±3	15±4†
HR (bpm)	67±3	67±4	61±2	61±2	68±2	66±2	65±2	61±3†
SEVR (%)	153±7	156±8	170±6	169±6	149±6	150±7	155±8	168±8†#
TTI (mmHg.sec/min)	2137±91	2090±87	1945±71	1904±78	2430±84 [^]	2345±93	2297±112 [^]	2098±93†
DTI (mmHg.sec/min)	3278±114	3179±136	3267±84	3183±103	3581±122	3457±97	3460±88	3465±116
AoTr (msec)	141±4	146±5	151±6	152±4	143±4	148±3	148±3	155±5
cfPWV (m/s)	6.59±0.35	6.86±0.45	6.34±0.25	6.06±0.27†#	7.45±0.44 ^{*^}	7.44±0.36	7.93±0.58 ^{*^}	7.24±0.44†#
crPWV (m/s)	8.29±0.32	7.97±0.37	8.33±0.32	7.65±0.39	7.76±0.22	7.64±0.24	7.93±0.40	7.98±0.36
clMT (mm)	0.61±0.03	0.63±0.03	0.58±0.02	0.58±0.02	0.69±0.06 [^]	0.67±0.04	0.70±0.04 ^{^*}	0.67±0.04
Dc (mm)	5.70±0.16	5.85±0.15	5.83±0.18	5.86±0.19	5.93±0.14	5.88±0.20	6.18±0.14	6.09±0.17
cCSA (mm ²)	10±1	11±1	10±1	10±1	12±1 ^{*^}	12±1	12±1 ^{*^}	12±1
CCA W/L ratio	0.21±0.02	0.21±0.02	0.20±0.01	0.20±0.01	0.23±0.02	0.23±0.02	0.22±0.01	0.22±0.01
CCA Strain (%)	8.0±0.9	7.9±0.7	7.2±0.9	7.5±0.6	6.7±0.5	7.0±0.5	7.3±0.6	7.9±0.8
CCA WS (mmHg)	20±1	19±1	17±1	17±1	24±3 [^]	23±2	22±2 [^]	21±2
CCA WT (kPa)	33±1	33±1	32±1	32±1	37±1 [^]	37±1	39±2 [^]	37±2

b=brachial; c = central; SBP, systolic pressure; DBP, diastolic pressure; PP, pulse pressure; P1 H, aortic incident pressure wave; AP, aortic augmentation pressure; AGI, aortic augmentation index; AGI@75HR, aortic augmentation adjusted for heart rate; HR, heart rate; SEVR Subendocardial viability ratio; TTI, Tension time index; DTI, Diastolic time index. AoTr, return of aortic reflective wave; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; Dc, carotid diameter in diastole; CCA W/L, common carotid artery wall to lumen ratio; CCA Strain, common carotid artery strain; CCA WS, common carotid artery circumferential wall stress, CCA WT, common carotid artery circumferential wall tension. FRS; Framingham Risk Score. Data presented as Mean ± SEM

*p<0.05 compared to healthy control NonT group; ^ p<0.05 compared to healthy control ExT group;

†p<0.05 compared to Pre-values within a group (Controls NonT, Controls ExT, MetS NonT, MetS-ExT)

#significant (p<0.05) group (e.g., MetS-NonT vs. MetS-ExT) by time (pre to post) interaction

Table 3: Differences in blood biomarkers pre and post intervention in MetS and controls

	Controls NonT		Controls ExT		MetS NonT		MetS ExT	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Total cholesterol, mg/dL	191±11	178±13	177±12	173±11	189±12	194±15	191±12	183±9
Triglycerides, mg/dL	93±14	86±10	81±17	90±17	161±23*^	173±18	115±16	130±17
HDL, mg/dL	56±5	52±5	57±4	53±4	40±4*^	38±3	50±5	47±4
Glucose, mg/dL	94±3	89±3	92±3	94±2	97±1	96±2	98±2	96±3
Insulin, µIU/mL	5.2±1.0	5.3±1.0	4.5±1.4	4.4±1.1	11.1±2.3*	11.8±2.3	9.8±1.4^	11.5±2.5
HOMA_IR	1.25±0.27	1.21±0.24	1.02±0.33	1.00±0.24	2.71±0.61*^	2.83±0.58	2.37±0.34^	2.68±0.57
sE-Selectin, ng/ml	0.78±0.09	0.75±0.07	0.57±0.06	0.56±0.02	0.91±0.10	0.87±0.09	0.95±0.01	0.83±0.01
sVCAM-1, ng/ml	7.7±1.8	8.6±1.9	6.4±1.4	5.5±1.2	7.3±1.4	7.2±1.3	6.0±1.5	5.5±1.3
sICAM-1, ng/ml	3.5±0.9	3.4±0.8	4.4±0.7	4.2±0.7	4.0±0.6	3.8±0.6	5.0±0.7	5.1±0.9
tPAI-1, ng/ml	1.2±0.1	1.1±0.1	1.2±0.3	1.0±0.2	1.4±0.2	1.4±0.2	1.2±0.2	1.3±0.2
IL-6, pg/ml	1.3±0.4	1.4±0.4	1.3±0.4	1.0±0.3	4.3±1.9	2.3±0.3	3.1±1.1	3.0±0.8
IL-8, pg/ml	3.2±1.0	3.7±1.0	2.8±0.6	2.4±0.5	5.1±0.8	4.8±1.2	4.1±0.9	4.0±0.6
TNFα, pg/ml	5.3±1.1	5.5±1.5	3.2±0.4	2.8±0.3	5.3±0.9	5.0±0.9	5.5±0.6	6.0±0.8
MMP-1, pg/ml	21±3	32±10	23±3	24±5	24±3	24±4	27±5	22±5†
MMP-2, pg/ml	3452±429	3484±508	3962±202	3962±442	4263±427	4260±326	4383±405	3793±143
MMP-7, pg/ml	648±61	629±60	705±139	674±138	543±48	571±64	893±215	789±202#
MMP-9, pg/ml	849±145	656±111	751±77	635±100	853±121	741±55	858±95	879±141
MMP-10, pg/ml	20±2	30±8	25±3	24±1	25±3	24±2	27±4	27±5
TIMP-1, pg/ml	1459±711	1173±456	1533±544	1569±555	1245±205	1323±222	2273±581	2302±664
TIMP-2, pg/ml	1585±836	1314±607	1794±655	1781±694	1290±233	1305±239	1824±470	1888±575

*p<0.05 compared to healthy control NonT group; ^ p<0.05 compared to healthy control ExT group;

†p≤0.05 compared to Pre-values within a group (Controls NonT, Controls ExT, MetS NonT, MetS-ExT)

#significant (p≤0.05) group (e.g., MetS-NonT vs. MetS-ExT) by time (pre to post) interaction

Sample size for examining the effects of exercise training on blood biomarkers is noted below:

Adhesion molecules: Con NonT n=6; Con ExT, n=9; MetS NonT n=9; MetS ExT, n=9

Inflammatory markers: Con NonT n=6; Con ExT, n=10; MetS NonT n=9; MetS ExT, n=9

MMP: Con NonT n=7; Con ExT, n=10; MetS NonT n=8; MetS ExT, n=10

Figure Legends

Figure 1. Example of central pressure waveform. The systolic and diastolic pressures are the peak and trough of the pressure waveform. Augmentation pressure (AP) is the pressure added to the forward wave by the reflected wave ($P_1 - P_2$), whereas augmentation index is the ratio between AP and central pulse pressure ($PP = \text{systolic} - \text{diastolic pressure}$). The dicrotic notch represents closure of the aortic valve and is used to calculate ejection duration (ED). Time to wave reflection is calculated at the point of rise in the initial ejection wave to the onset of the reflected wave. Travel time (Tr) represents the time taken for the forward pressure wave from the aorta to the reflection site and back. Systolic-to-diastolic pressure shifts were assessed by the systolic (red shaded area) and diastolic (blue shaded area) pressure-time integrals.

Figure 2. Relationship between peak aerobic capacity (VO_2 peak) and arterial markers, namely carotid femoral pulse wave velocity (A), carotid intima medial thickness (B), augmentation pressure (C), and brachial (bSBP) and central (cSBP) systolic pressure (D).

Figure 3. Relationship between the change (post- pre values) in peak aerobic capacity (VO_2 peak) and carotid femoral pulse wave velocity.

Figure 1.

Figure 2.

Figure 3.

Chapter 4

IMPROVED ARTERIAL-VENTRICULAR COUPLING IN METABOLIC SYNDROME AFTER EXERCISE TRAINING - A PILOT STUDY

Sara B. Fournier^{1,2}, David A. Donley¹, Daniel E. Bonner¹, Evan DeVallance^{1,2}, I. Mark Olfert^{1,2}, and Paul D. Chantler^{1,2}

¹*Division of Exercise Physiology, School of Medicine, West Virginia University;* ²*Center for Cardiovascular and Respiratory Sciences, School of Medicine, West Virginia University*

Running Head: Arterial Ventricular Coupling and Exercise

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Abstract

Purpose: The metabolic syndrome (MetS) is associated with a three-fold increase risk of cardiovascular (CV) morbidity and mortality, which is in part, due to a blunted CV reserve capacity, reflected by a reduced peak exercise left ventricular contractility and aerobic capacity, and a blunted peak arterial-ventricular coupling. To date, no study has examined whether aerobic exercise training in MetS can reverse the peak exercise CV dysfunction. Further, examining how exercise training alters CV function in a group of individuals with MetS prior to the development of diabetes and/or overt CVD, can provide insights into whether some of the pathophysiological changes to the CV can be delayed/reversed, lowering their CV risk. The objective of this study was to examine the effects of 8 weeks of aerobic exercise training in individuals with MetS on resting and peak exercise CV function.

Methods: Twenty MetS underwent either 8 weeks of aerobic exercise training (MetS-ExT; n=10) or remained sedentary (MetS-NonT; n=10) during this time period. Resting and peak exercise CV function was characterized using Doppler echocardiography and gas exchange.

Results: Exercise training did not alter resting left ventricular diastolic or systolic function and arterial-ventricular coupling in MetS. In contrast, at peak exercise an increase in LV contractility (40%, $p<0.01$), cardiac output (28%, $p<0.05$) and aerobic capacity (20%, $p<0.01$), while a reduction in vascular resistance (30%, $p<0.05$) and arterial-ventricular coupling (27%, $p<0.01$), were noted in the MetS-ExT but not the MetS-NonT group. Further, an improvement in Lifetime Risk Score was also noted in the MetS-ExT group.

Conclusions: These findings have clinical importance as they provide insight that some of the pathophysiological changes associated with MetS can be improved and lower the risk of CVD.

Key Words: cardiovascular disease, cardiac function, arterial function, and obesity.

Introduction

The metabolic syndrome (MetS) is a cluster of metabolic risk factors (abdominal obesity, elevated blood pressure and glucose, and dyslipidemia) that exerts a three-fold increased risk of cardiovascular disease (CVD) mortality compared to non-MetS individuals [1]. Alarming, the prevalence of MetS in US adults is 34% and is on the rise due, in part, to rising rates of obesity [2]. MetS has become a leading health concern because of its strong association with future myocardial/cerebral vascular events and CVD mortality [1].

The MetS-associated pathophysiological changes to the CV system contribute to the increased CVD risk. Such changes include an increase in arterial dysfunction (increased arterial stiffness, and endothelial dysfunction), left ventricular (LV) hypertrophy, and impaired CV reserve capacity [3-5]. In particular, individuals with MetS demonstrate an impaired coupling between the heart and arterial system [5]. This interaction, termed arterial-ventricular coupling (E_a/E_{es}), is an important determinant of net CV performance [6] and cardiac energetics [7]. E_a/E_{es} is indexed by the ratio of effective arterial elastance (E_a ; a measure of the net arterial load imposed on the left ventricle) to left ventricular end-systolic elastance (E_{es} ; measure of LV chamber performance) [6]. Impaired E_a/E_{es} is most readily evident when the CV system is under stress and/or with increasing age [8]. For example, in MetS, the ability to reduce the E_a/E_{es} ratio is blunted during aerobic exercise [5]. Further, this impaired coupling response coincides with a reduced peak aerobic capacity in MetS [5], which has been proposed to be an independent predictor of mortality relative to other risk factors [9].

In healthy older individuals, exercise training has been shown to increase aerobic capacity, improve peak LV performance (smaller end-systolic volume and increased stroke volume), and increase LV contractility [10, 11]. In patients with coronary artery disease, Ea/Ees during handgrip isometric exercise was improved after exercise training. It is unknown whether exercise training in people with MetS (prior to the development of diabetes or overt CVD) can improve peak exercise LV-arterial coupling and CV function. An improvement in Ea/Ees and CV function in MetS would likely reflect a lower CVD risk. Thus, the objective of this study was to examine the effects of 8 weeks of exercise training on peak exercise arterial-ventricular coupling in clinically defined MetS patients without diabetes or clinical indications of CVD. We hypothesized that aerobic exercise training would improve peak Ea/Ees in MetS due to an improvement in peak exercise LV contractility.

Methods

Study Population

Twenty MetS subjects free from overt CVD and diabetes as determined by a detailed medical history, physical examination, and a normal resting and exercise electrocardiogram participated in this study. MetS was defined according to the updated National Cholesterol Education Program: Adult Treatment Panel III comprised of three out of the following five components: 1) obesity (waist men >102 cm, women >88 cm); 2) low HDL cholesterol (men <40 mg/dL; women <50 mg/dL); 3) hypertriglyceridemia (≥ 150 mg/dL); 4) elevated glucose (≥ 100 mg/dL & <126 mg/dL), and; 5) elevated BP (130/85 mmHg or use of hypertensive medications).

Exclusion criteria included diabetes mellitus (HbA1c ≥ 6.5 % or use of diabetic medications), pulmonary disease, angina, atrial fibrillation, aortic stenosis, anemia, myocardial infarction, stroke, or coronary revascularization as assessed by a detailed medical history physical examination and a resting and exercise electrocardiogram. Subjects who participated in regular exercise, defined as >30 minutes, 3 times/week were excluded. All subjects provided written informed consent to participate that was approved by WVU Institutional Review Board.

Study Design

Physiological assessments were performed between 7:00-10:00 AM, in a quiet, temperature-controlled room, after a 12 hour fast and abstinence from alcohol, caffeine, and vitamins. CV medications were withheld 24 hours prior to assessments. After a

minimum 15 minutes of quiet rest subjects underwent resting non-invasive assessments of arterial and cardiac structure and function.

Exercise Performance

Following assessment of resting CV measurement, subjects exercised to exhaustion on a modified cycle ergometer (Monarch 827, Sweden) equipped with a car seat to allow semi-recumbent exercise in seated upright position at approximately 130 degrees. This upright position is necessary and important to allow acquisition of optimal echocardiographic images during exercise. Throughout exercise the echo images were acquired approximately 60-90 seconds into each 3-minute exercise workload. If all images were not acquired within the time frame the duration of the exercise stage was extended to acquire those images. Pedal speed was maintained at 50 rpm, and workloads increased by 25 W every 3 minutes until exhaustion. Oxygen consumed (VO_2), carbon dioxide produced (VCO_2), respiratory exchange ratio ($\text{RER}=\text{VCO}_2/\text{VO}_2$), ventilation (Ve), ventilatory threshold (VT), and the ventilatory equivalent for CO_2 (Ve/VCO_2) were measured throughout exercise using a metabolic cart (TureOne 2400, ParvoMedics, Sandy, UT). Subjective symptoms of fatigue (BORG score 6 to 20), and blood pressure (sphygmomanometry) were recorded at the end of each workload.

Rest and Exercise Echocardiography

Echocardiograms were obtained using a GE Vivid i (GE Healthcare, Chalfont St. Giles, United Kingdom), portable ultrasound imaging system with a 5S-RS (2.0-5.0 MHz) Wideband Phased Array transducer. All echocardiograms were performed by

experienced registered diagnostic cardiac sonographers. At rest, standard 2-dimensional images were obtained in the following acoustic views; parasternal long axis, and apical 4 chamber view. Pulsed wave Doppler tracings of the mitral valve inflow velocity (recorded at the leaflet's tips) were recorded in the apical 4 chamber view. Continuous/pulse wave Doppler tracings of the LV outflow tract velocity were obtained in the apical 5 chamber view positioned 5 mm proximal to the aortic valve. Spectral tissue Doppler imaging was performed in the apical 4 chamber view with the gate sample positioned in the lateral corner and septal side of the mitral annulus. During exercise, the sonographer quickly acquired a 2-dimensional image of the parasternal long axis view to obtain the size of the LV outflow tract diameter (base of the aortic leaflets). The sonographer then focused on capturing: 4-chamber views to obtain cardiac volumes and mitral flow velocities; and 5-chamber views to obtain pulse-and continuous-wave Doppler-flow from the LV outflow tract.

Left Ventricular Geometry and Remodeling

In the supine position, LV dimensions, wall thickness, and chamber volumes were determined in triplicate from 2-dimensional, M-mode, and Doppler spectra echocardiography using standard methods [12]. Sex-specific LV hypertrophy (LVH) and geometry patterns, based on LV mass index and relative wall thickness were defined as LV mass index $>95 \text{ g/m}^2$ for women or $>115 \text{ g/m}^2$ for men, and LV geometry was classified as normal, concentric remodeling, concentric LVH, or eccentric LVH [12].

Arterial-Ventricular Measurements

In the upright, seated rest position and during exercise, LV end diastolic (EDV) and end-systolic (ESV) volumes, along with ejection fraction (EF) were determined from Simpson's biplane method [12]. Cardiac volumes were normalized to body surface area (BSA). Cardiac index (Ci) was determined from the product of heart rate (HR) and stroke volume index. Peak arteriovenous oxygen extraction was calculated from the Fick equation ($VO_{2peak}/\text{cardiac output}$). Systemic vascular resistance index (SVR_i) was calculated as $\text{mean arterial pressure (MAP)} \times 80 / \text{Ci}$. The indexes of arterial and ventricular elastance were calculated as; 1) arterial elastance (E_a) a measure of the net arterial load, was calculated as $\text{end-systolic pressure (ESP)} / \text{stroke volume (SV)}$, where ESP is approximated as $0.9 \times \text{systolic blood pressure (SBP)}$ [6]. Of note, ESP calculated as $0.9 \times \text{SBP}$ has previously been shown to closely approximate central ESP [13]; 2) LV end-systolic elastance (E_{es} [calculated from BP, stroke volume, EF, and pre-ejection and systolic ejection time intervals from LV outflow Doppler]), was determined by the validated single-beat technique [14]; and 3) Arterial ventricular coupling ratio was determined from E_a/E_{es} [6]. Of note, E_a/E_{es} is mathematically related to EF via the formula $E_a/E_{es} = (1/EF) - 1$. Reserve was defined as the difference in these variables between seated rest and peak exercise.

Diastolic Function

In the supine position, the medial mitral annular early diastolic velocity (e') was determined by spectral tissue Doppler imaging (GE Vivid i) using standard methods. Early (E-wave) and late (A-wave) transmitral flow velocities, the isovolumetric relaxation time (IVRT), and the deceleration time of early filling velocity (Dec T) were measured by

pulsed-wave Doppler (GE Vivid i). End-diastolic pressure was estimated as $EDP = 11.96 + 0.596 \times E/e'$ [15]. Because of the high incidence of fusion of the E- and A-wave during moderate/high intensity exercise, with the A-wave dominating, we are not able to measure Dec T- or E-wave. Therefore we were limited to measuring peak exercise IVRT and A-wave.

Body Anthropometry

Height, weight, and waist and hip circumference were measured using standard laboratory procedures. Fat distribution was assessed by measuring the waist circumference at the site of the smallest circumference between the rib cage and the iliac crest with subjects in a standing position. Hip circumference was measured at the site of the largest circumference between waist and thighs. Lean body mass and fat mass were measured using air displacement plethysmography (BodPod, COSMED Inc., USA). During assessment of body composition subjects wore close-fitting bathing suits and a swim cap. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

Blood Analyses

Venous blood sampling was obtained in the morning after a 12-hour overnight fast. Post training venous samples were collected at least 48 hours after the last exercise session. Plasma obtained from blood sampling was analyzed at West Virginia University Hospital's central laboratory in Morgantown, West Virginia. Total cholesterol,

high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were determined in plasma (lithium heparin, Becton Dickinson Plasma Separator Tubes) using Beckman-Coulter (CA, USA) DxC automated chemistry analyzers. Total cholesterol was measured using a cholesterol esterase/cholesterol oxidase/peroxidase-driven, timed-endpoint method (Coefficients of Variation <7%). HDL cholesterol was measured using a cholesterol esterase/cholesterol oxidase/peroxidase-driven, timed endpoint assay with automated initial homogeneous solubilization step (Coefficients of Variation <7%). Triglycerides were measured using a lipase/glycerol kinase/glycerophosphate oxidase/horseradish peroxidase-driven, timed-endpoint method (Coefficients of Variation 5-7%). Glucose was measured electrochemically using a glucose oxidase/catalase/molybdate-driven oxygen rate method (Coefficients of Variation <5%). Glycated hemoglobin (A1c fraction) was measured in whole blood (K₂-EDTA, Becton Dickinson) using a BioRad (Hercules, CA, USA) Variant II Turbo High Performance Liquid Chromatography (HPLC) system (Coefficients of Variation <2%). Insulin was measured in serum (untreated/red-top tube, Becton Dickinson) on an Immulite 2000 immunochemistry system (Siemens, USA; Coefficients of Variation <10%). Homeostasis model assessment of insulin resistance (HOMA-IR) was estimated with the following formula: $\text{insulin resistance} = \text{fasting plasma insulin (in microunits per millileter, } \mu\text{U/mL)} \times \text{fasting plasma glucose (FPG, in millimoles per liter, mmol/L)} / 22.5$.

Training Intervention

MetS were assigned into either an 8 week aerobic exercise intervention group (MetS-ExT) or an 8 week non-exercise control (MetS-NonT) group. Group assignments

were made using a pseudo-random balance approach to ensure equal numbers enrolled in each group. The MetS-NonT exercise group (n=10; 50% female) were asked to maintain their normal sedentary lifestyle. The MetS-ExT group (n=10; 70% female) performed 8 weeks of supervised aerobic exercise in the Human Performance Lab at West Virginia University School of Medicine, 3 times per week for 60 minutes at a fixed exercise intensity. The intensity of prescribed exercise was based on individual results of maximal cardiopulmonary exercise tests. We used a ramp exercise protocol, whereby exercise training intensity started at 60% of heart rate reserve (heart rate range determined during exercise stress test) and increased every 2 weeks by 10%; from weeks 6-8 heart rate reserve was set at 85%. Adherence to the exercise prescription was documented through the use of wrist watch-style heart rate monitors (E600, Polar Electro OY, Oulu, Finland) and physical activity logs. Approved modalities included treadmills, elliptical machines, and cycle ergometers. All participants were instructed to maintain current eating behaviors for the duration of the 8-week intervention. All post-training measurements were performed 24-48 hours after the last exercise training session to avoid the immediate effects of a single bout of exercise. Measurements made before and after exercise training were obtained at the same time of day for each subject.

Lifetime Risk Score for CVD

The Lifetime Risk Score is a strong predictive capacity for future CV mortality and is based on an algorithm that incorporates sex (male/female), age (years), SBP (mmHg), diabetes mellitus (yes/no), total cholesterol (mg/dL), smoking (yes/no), BMI

(kg/m²), and physical fitness (Metabolic equivalent; 1 MET = 3.5 ml/kg/min) [16, 17]. Calculation of the score is available using a web-based interface (www.lifetimerisk.org).

Statistical Analysis

Measurements of CV function were performed offline by a single investigator who was blinded to group allocation. The intra-class correlation coefficient (ICC) and coefficient of variations for all echocardiographic variables were derived in a subset of subjects (n=8). At rest, the ICC and the coefficient of variation for all variables, collected on two separate days, was >0.80 or between 7-12%, respectively. Similar results were obtained for echocardiographic variables evaluated during peak exercise with all variables having an ICC>0.80 and coefficient of variation between 7-12% with the exception of the arterial-ventricular coupling ratio (ICC=0.63).

Normality was evaluated by the Kolmogorov-Smirnov test. Continuous variables were log transformed as necessary. To evaluate the effects of exercise training, paired *t*-tests and two-way repeated-measures ANOVA were used. We also ran a mixed effects models with a time-varying covariate to examine whether the change (pre vs post training intervention) in CV parameters were due to changes other CV parameters. All analyses were performed with the statistical package SPSS version 21 (SPSS, Chicago, IL). Values shown in the tables represent means $\pm$ SEM unless otherwise stated. A $p \leq 0.05$ was defined as significant.

Results

Age, anthropometric, and metabolic characteristics of MetS individuals are shown in Table 1. The breakdown of metabolic components were: 100% had a waist circumference >102 cm (men) or >88 cm (women); 75% had elevated BP (130/85 mmHg or use of hypertensive medications); 70% had a low HDL cholesterol (men <40 mg/dL; women <50 mg/dL); 30% had hyper-triglyceridemia (≥ 150 mg/dL); and 50% had elevated glucose (≥ 100 mg/dL). In terms of LV remodeling, 15% had normal LV geometry and concentric remodeling, 50% had eccentric LV hypertrophy, and 20% had concentric LV hypertrophy.

Effects of Exercise Training on Metabolic Profile, Body Composition, and LV Geometry

Eight weeks of exercise training did not alter basal fasting HbA1C, glucose, insulin, HDL, triglycerides, or HOMA-IR (Table 1). Further, exercise training was insufficient to significantly alter body composition (weight, lean mass or percent body fat), LV mass (in absolute terms and relative to BSA), the internal LV dimensions (internal diameter, septal and posterior wall thickness), and relative wall thickness in MetS (Table 2).

Effects of Exercise Training on LV Diastolic Function

Resting LV diastolic function (i.e., E-wave, A-wave, E/A ratio, IVRT, Dec T, E/e' and EDP) was not affected by 8 weeks of exercise training in MetS (Table 2). During exercise we were limited to examining changes in components of LV diastolic function namely IVRT, and A-wave. No significant differences in peak IVRT (MetS-ExT; 16 ± 1 vs.

16±2; Non-T; 15±1 vs. 15±2), and A-wave (MetS-ExT; 1.38±0.06 vs. 1.28±0.11; Non-T; 1.48±0.08 vs. 1.53±0.06) were found in either MetS-ExT or Non-T pre and post intervention.

Effects of Exercise Training on Arterial Ventricular Coupling

Exercise training in MetS did not alter resting Ea/Ees, Ea, or Ees (Figure 1). Further, with the exception of resting SVR_i which was lower in the MetS-NonT, no differences in resting CV function were evident in either MetS group (Table 3). There were no significant time (pre and post) by group (MetS-ExT vs. NonT) interactions at rest for any CV parameter.

At peak exercise, aerobic training in MetS lowered peak Ea/Ees (-27%, $p<0.01$) by increasing peak Ees (+40%, $p<0.01$) and no change in peak Ea in MetS-ExT (Figure 1). In contrast, peak Ea/Ees, Ea, and Ees did not significantly differ between pre and post visits in MetS-NonT. Similarly, peak EF, and Ci were significantly ($p<0.05$) increased, and peak ESV_i and SVR_i were reduced ($p<0.05$) after exercise training in MetS-ExT, whereas no differences were found in MetS-NonT (Table 4). Furthermore, significant time (pre and post) by group (MetS-ExT vs. NonT) interactions for peak Ea/Ees, Ees, EF, and SVR_i were evident.

Exercise capacity, in both absolute (L/min) and relative (ml/min/kg of body mass or lean mass) terms was improved in MetS after 8 weeks of exercise training as reflected by a 19-20% increase ($p<0.01$) in VO_{2peak} and a 4% increase ($p<0.05$) in Ve/VCO_2 (Table 4). Of note, a time (pre and post) by group (MetS-ExT vs. NonT) interaction for VO_{2peak} was identified despite no differences in the respiratory exchange

ratio, or perceived rate of exertion, in either MetS group. Further, no differences in peak arteriovenous oxygen difference were evident pre or post intervention in MetS (Table 4). Taking all individuals into consideration, an initial relationship between pre-intervention values for Ea/Ees delta (max-rest) and VO_{2peak} ($r=-0.58$, $p<0.01$) was found. We then examined whether the difference in Ea/Ees delta from visit 1 and visit 2 was correlated with the change from visit 1 and 2 in VO_{2peak} , however this relationship did not reach statistical significance ($r=-0.40$, $p=0.09$). In a stepwise mixed model approach with repeated measures and a time-varying covariate, we found that Ea/Ees delta contributed towards 16% of the variance on the effects of exercise training on peak VO_2 .

Lifetime Risk Score

In the MetS-ExT group, the Lifetime Risk Score was significantly reduced ($20\pm7\%$ to $16\pm6\%$, $p=0.01$) after training, whereas in the MetS-NonT group no difference was found ($13\pm2\%$ vs. $13\pm2\%$, $p=0.8$). Further, we identified a significant inverse correlation between the Lifetime Risk Score and peak VO_2 ($r=-0.53$, $p=0.02$).

Discussion

This is the first study to examine the effects of aerobic exercise training on peak arterial-ventricular coupling in patients with the MetS. We report that following 8 weeks of aerobic exercise training, peak arterial-ventricular coupling, peak LV contractility and aerobic capacity were significantly improved and similar to levels noted in healthy untrained controls [5]. Our unique patient population afforded the opportunity to assess whether exercise training can reverse impaired CV function reported in MetS patients prior to a diagnosis of CVD and/or diabetes. Since MetS is believed to be a harbinger in the pathogenesis of diabetes and CVD, these data provide evidence that exercise based interventions at this critical (preclinical disease) time point may help to reverse the pathophysiological progress of CVD, and/or delay the progression to overt CVD and/or diabetes.

The MetS is associated with pathophysiological changes to the CV system associated with an increased CVD mortality risk. Recently we reported that resting LV systolic function was well preserved in MetS patients who clearly manifested the symptoms of the metabolic disorder (including hypertension, insulin resistance, and hyperlipidemia) but who are free from overt CVD and/or diabetes [5]. Conversely, in this same population we observed LV remodeling, LV hypertrophy and reduced LV diastolic function at rest [5]. The progression from MetS to T2DM is characterized by additional LV enlargement and LV diastolic dysfunction, with evidence of LV systolic dysfunction and related sympathetic alterations [18]. In our MetS population, exercise training was unable to reverse the LV structural changes or impairments in LV diastolic function. The lack of an observed effect of exercise training on LV mass is likely due to the duration of

the exercise stimulus (8 weeks). Indeed, an intervention consisting of several months of aerobic training was found to be sufficient to exert physiological LV remodeling [19], reduce LV wall thickness, and reduced LV wall thickness-to-radius ratio [20]. To what extent exercise training can improve LV diastolic function by improving the compliance of the heart remains controversial. Two recent studies examining a full year of exercise training, in MetS patients without T2DM or CVD [21] and healthy senior individuals [19], found no significant improvements in resting LV diastolic function. Advancing age and the development of CVD induce structural and functional alterations to the heart that is reflected by a reduction in the number of cardiomyocytes, an increase in connective tissue volume, and an increase in the formation of advanced glycation end products, which collectively result in impairment of LV diastolic function [22]. Data would suggest that once these cross-linked collagen proteins are formed they are pathologically irreversible. Thus, any potential improvements in LV diastolic function to be gained from exercise training may be limited by cross-linked collagen [19].

The coupling between the heart and arterial system is an important and largely underappreciated determinant of cardiac performance and energetics [6]. At rest, the coupling ratio is well maintained around 0.7-1.0 to optimize CV efficiency. This ratio is preserved with advancing age and in patients with heart failure [8, 23]. Indeed, we have also recently shown that resting Ea/Ees is around 0.9 in MetS patients [5]. In this study, exercise training in MetS did not alter resting Ea/Ees, or its components. While resting Ea/Ees is fairly well conserved, the ability of the CV system to respond to stress, in particular exercise, is blunted with age, and in heart failure patients [8, 24]. We have previously shown that the Ea/Ees response to exercise is significantly impaired in MetS

patients [5], which is, in part, due to impaired LV contractility and a blunted peripheral vasodilatory response to exercise [5]. Importantly, this study indicates that 8 weeks of exercise training is sufficient to increase the peak LV contractile response and improve peak exercise Ea/Ees in MetS patients, as direct result of improved peak Ees.

Although we have shown that arterial-ventricular coupling is improved after exercise training in middle-aged individuals with MetS, it is also important to identify whether older individuals with MetS would have the same beneficial effects of exercise training. In older (age >60 years) healthy individuals, peak exercise cardiac function is typically improved, at least up to the 7th decade, after exercise training depicted by increases in Ci, SV, EF, LV contractility, and VO_{2peak} [25-28]. Future research should establish to what extent older individuals with MetS demonstrate improvements in arterial and cardiac function after exercise training. This is especially important given that the presence of MetS with older age is often accompanied by CVD (i.e., T2DM, coronary artery disease, etc.), and with increased formation of cross-linked advanced glycation end products in the LV and arterial walls, which may limit the physiological responses to exercise training [29].

A blunted Ea/Ees response during exercise has been shown to correspond with a decrease in VO_{2peak} in MetS [5] and heart failure [24] patients. An inverse relationship has also been reported between VO_{2peak} and Ea/Ees during exercise [30], which was confirmed in our study. In addition, acute infusion of verapamil improved arterial-ventricular coupling and resulted in a corresponding improvement in exercise capacity [31]. These data suggest a direct link between Ea/Ees and aerobic capacity, in that an improvement in the coupling likely results in a more effective transfer of blood from the

heart to periphery thereby increasing functional reserve capacity. Indeed, in the presence of a stiffer heart there is a greater change in BP for a given change in volume, which in a closed-system, can amplify the BP response and impair net cardiac ejection [31]. In the current study, we found an increase in peak SV without a significant change in BP suggesting a more compliant cardiac response after exercise training. Further, the improvement in peak Ea/Ees was accompanied by a 20% increase in VO_{2peak} , and that the change in Ea/Ees seemed to contribute ($\approx 16\%$) towards the improved VO_{2peak} . Given that aerobic capacity is determined by both central (HR and SV) and peripheral factors (skeletal muscle mass, calcium cycling, mitochondria capacity, capillary density, etc.), it is likely that the improved coupling, along with other physiological adaptations that were not examined in our study, contributed towards the improved VO_{2peak} in MetS after exercise training. Indeed, Tjonna et al. [32] observed a 16% and 36% increase in VO_{2peak} after 16 weeks of either continuous aerobic exercise or high intensity aerobic interval training in MetS, respectively. It was suggested that enhanced skeletal muscle capacity (improved calcium cycling and mitochondria capacity) contributed to the exercise-induced improvement in VO_{2peak} [32]. Although, we did not directly measure skeletal muscle oxidative capacity or oxygen extraction, these responses to exercise training have been reported elsewhere [32, 33]. In contrast to these findings, we did not observe an improvement in peak arteriovenous oxygen difference (estimated from the Fick equation) after 8 weeks of exercise training in MetS, suggesting that muscle adaptation played a minor role in the reported improvements in aerobic exercise capacity in our patients. The effects of exercise training on improving peak arteriovenous oxygen difference in other populations are mixed with some reporting no

changes [19, 28] and others showing an increase [26, 34]. This lack of change may be related to the intensity of exercise training, whereby an increase arteriovenous oxygen difference was reported after high-intensity exercise training, but not after low-intensity training [26]. An increase in exercise intensity and duration may be required to increase peripheral oxygen extraction in individuals with MetS. Further, research is needed to clarify the relationship between Ea/Ees, and $\text{VO}_{2\text{peak}}$.

The improvement in LV contractility during exercise may have been due, in part, to an increased stroke volume, a decreased afterload, and improved arterial-ventricular coupling. Indeed, improvements in peak exercise SVi and ESVi were reported in MetS after exercise training. Similar improvements in cardiac volumes have been reported after exercise training in older sedentary individuals [10, 19]. Improved peak LV performance post exercise training is unlikely to be due to enhanced myocardial β -adrenergic responses, as chronic exercise training has not been reported to alter β -adrenergic function [35]. However, exercise training has been shown to improve calcium handling in experimental animal models, thereby improving cardiomyocyte function [36]. Accordingly, improvements in calcium handling may have contributed to the improvement in LV contractility noted in MetS. The enhanced ability of the LV to empty as fitness increases may relate to a reduction in arterial stiffness/afterload in the conditioned state [37]. However, following 8 weeks of exercise training, no improvements in Ea during exercise were reported in MetS. In contrast, a significant reduction in peak vascular resistance was established in the exercise trained MetS patients. Ea is an integrative index that incorporates the principal elements of arterial load, including systemic vascular resistance, total arterial compliance, characteristic

impedance, and systolic and diastolic time intervals. Ea is therefore regarded a measure of the net arterial load that is imposed on the LV [6]. Thus, the lack of change in Ea does not necessarily indicate that specific components of arterial load were not improved at peak exercise after training. This was clearly evident with the improvement in peak SVR_i. Further, the improvement in SVR_i or the lack of change in Ea, were not attributed to the slight changes in peak HR noted in the control group, as evident by similar findings after adjusting for HR as a time-vary covariate in a mixed effects model. Whether the improvement in SVR_i at peak exercise is due to release of vasodilators causing vascular relaxation remains to be elucidated. Improvements have also been noted in resting endothelium-dependent vasodilatation in obese and MetS patients after exercise training [32]. Accordingly, despite the lack of change in Ea, peak arterial function may have been improved in MetS, thus contributing to an improved aerobic capacity.

The beneficial CV effects at peak exercise attributed to exercise training were not a reflection of a change in body fat or lean mass. However, numerous studies have reported that regular moderate intensity exercise can result in reductions in weight and fat mass [38, 39]. Thus, the lack of change in body composition in our study is likely due to the short duration of exercise training (8 weeks). Further, clinical metabolic biomarkers (HDL, triglycerides, glucose, etc.) remained unchanged. Although we did not find improvements in body composition or clinical blood biomarkers after training in MetS, we believe that improvements in peak CV function and aerobic capacity reflect a reduction in CV risk in MetS patients. A strong association exists between aerobic capacity and mortality with a positive correlation between improvements in aerobic

capacity and an improved prognosis [40]. A relationship that seems to be more robust than the relationship between weight loss and mortality [41]. For example, for each MET (3.5 ml/kg/min) increase in exercise capacity confers a 12% improvement in survival [9]. The average increase in peak VO_2 (ml/kg/min) in the MetS-ExT group was 3.2 ml/kg/min, suggesting an improvement in survival. Further, the Lifetime Risk Score in MetS-ExT was significantly reduced by 4%, and the Lifetime Risk Score was inversely correlated with peak VO_2 . However, to fully prevent the progression to overt CVD and/or diabetes, and the pathophysiological changes to the CV system that accompanies this transition, persistent physical activity in combination with a nutritional dietary regimen (that includes optimal vitamin/mineral consumption) is required.

Study Limitations

There are several limitations. First, the sample size for our training and non-training group is modest ($n=10$ in each). Although we find significant differences in peak Ea/Ees, this was due principally to a significant change in peak Ees ($p<0.05$ with statistical power >0.75), but we were underpowered in our statistical power for Ea (power=0.10). While it is evident that we have sufficient power in those CV variables where significance was observed, we cannot exclude the possibility of a type II statistical error (i.e. that we have falsely accepted the null hypothesis) in the CV variables that we not-significantly different where power was low. Therefore, these data should be regarded as preliminary until we, or others can obtain data on a larger population sample. In the current study, we examined the effects of exercise training on Ea (net arterial load) as our arterial function parameter. Although it is important to study

the interaction between the heart and arterial system in the same domain (i.e., elastance), measuring specific aspects of arterial function such as characteristic impedance, arterial stiffness (via pulse wave velocity), and intima medial thickness would provide additional insights into the beneficial effects of exercise training in MetS. Further, sex differences in the effects of exercise training on LV stiffness may have gone undetected given the small number of male vs. female subjects. Future research should examine whether there are sex-related differences in the coupling response, at rest and during exercise, after exercise training.

Second, pressure and flow were not directly measured, but rather estimated from non-invasive surrogates. However, the methods we used have been previously validated against invasive hemodynamic measurements [14]. Our peak cardiac data may be underestimated due to a systematic underestimation of LV volumes from 2-D echocardiography [42, 43] and the challenge of acquiring echocardiographic images during exercise. However, the technique we used has been successfully used by others [24, 44], and similar values have been observed suggesting fidelity in our data.

Third, we were limited in our ability to comprehensively characterize the extent of diastolic function during exercise because the focus of this study was to examine the impact of exercise training on peak exercise E_a/E_{es} and LV systolic function, and therefore echocardiographic views were optimized to examine systolic function. Further, the acquisition of LV diastolic parameters during exercise is challenging. Thus, we cannot rule out that exercise training in MetS also improved peak exercise LV diastolic function.

Fourth, peak arteriovenous oxygen difference was not measured but rather it was estimated using the Fick equation (VO_2 divided by cardiac output). The Fick technique has been used to calculate arteriovenous oxygen difference in number of recent physiologic studies investigating mechanisms of exercise intolerance [45, 46]. Our peak arteriovenous oxygen difference values were somewhat higher than reported by others [45], possibly due to underestimation of cardiac output. Most importantly, because key variables were measured at all testing times using identical methods in both groups, and because we assessed changes in reserve capacity (peak values – resting values) within individuals, comparisons of cardiac output and estimated arteriovenous oxygen difference between groups are valid.

Lastly, the short duration of exercise training (8 weeks) may have been insufficient to alter cardiac structure, body composition, and metabolic blood biomarkers. Thus, longer exercise training programs that incorporate different exercise modalities (interval, aerobic, and resistance training) are important to fully understand the role that exercise training has on improving CV structure/function in patients with MetS.

Conclusion

In conclusion, 8 weeks of aerobic exercise training of moderate-to-high intensity significantly improved peak exercise arterial-ventricular coupling, LV contractility, peripheral vascular resistance, and aerobic capacity in MetS individuals without overt CVD and Type 2 Diabetes. However, no improvements were evident in resting LV

structure and diastolic function, metabolic profile or body composition after exercise training.

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Disclosures

All authors report that there are no conflicts of interest, financial or otherwise in connection with the submitted article to disclose. The results of the present study do not constitute endorsement by ACSM.

Reference List

1. Malik, S., et al., *Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults*. Circulation, 2004. **110**(10): p. 1245-1250.
2. Mozumdar, A. and G. Liguori, *Persistent Increase of Prevalence of Metabolic Syndrome Among U.S. Adults: NHANES III to NHANES 1999–2006*. Diabetes Care, 2011. **34**(1): p. 216-219.
3. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. **43**(8): p. 1388-95.
4. Pagé, A., et al., *Metabolic Syndrome Is Associated With More Pronounced Impairment of Left Ventricle Geometry and Function in Patients With Calcific Aortic Stenosis: A Substudy of the ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin)*. Journal of the American College of Cardiology, 2010. **55**(17): p. 1867-1874.
5. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. Experimental Physiology, 2013. **99**(1): p. 149-163.
6. Chantler, P.D., E.G. Lakatta, and S.S. Najjar, *Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise*. Journal of Applied Physiology, 2008. **105**(4): p. 1342-1351.
7. Chantler, P.D., et al., *The sex-specific impact of systolic hypertension and systolic blood pressure on arterial-ventricular coupling at rest and during*

- exercise*. American Journal of Physiology-Heart and Circulatory Physiology, 2008. **295**(1): p. H145-H153.
8. Najjar, S.S., et al., *Age and gender affect ventricular-vascular coupling during aerobic exercise*. J Am Coll Cardiol, 2004. **44**(3): p. 611-7.
 9. Myers, J., et al., *Exercise Capacity and Mortality among Men Referred for Exercise Testing*. New England Journal of Medicine, 2002. **346**(11): p. 793-801.
 10. Stratton, J., et al., *Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men*. Circulation, 1994. **89**(4): p. 1648-1655.
 11. Ehsani, A., et al., *Exercise training improves left ventricular systolic function in older men*. Circulation, 1991. **83**(1): p. 96-103.
 12. Lang, R.M., et al., *Recommendations for chamber quantification*. Eur J Echocardiogr, 2006. **7**(2): p. 79-108.
 13. Kelly, R.P., et al., *Effective arterial elastance as index of arterial vascular load in humans*. Circulation, 1992. **86**(2): p. 513-21.
 14. Chen, C.H., et al., *Noninvasive single-beat determination of left ventricular end-systolic elastance in humans*. J Am Coll Cardiol, 2001. **38**(7): p. 2028-34.
 15. Ommen, S.R., et al., *Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study*. Circulation, 2000. **102**(15): p. 1788-94.
 16. Berry, J.D., et al., *Lifetime Risks of Cardiovascular Disease*. New England Journal of Medicine, 2012. **366**(4): p. 321-329.

17. Berry, J.D., et al., *Lifetime risks for cardiovascular disease mortality by cardiorespiratory fitness levels measured at ages 45, 55, and 65 years in men: The cooper center longitudinal study*. Journal of the American College of Cardiology, 2011. **57**(15): p. 1604-1610.
18. Straznicky, N.E., et al., *The relation of glucose metabolism to left ventricular mass and function and sympathetic nervous system activity in obese subjects with metabolic syndrome*. J Clin Endocrinol Metab, 2013. **98**(2): p. E227-37.
19. Fujimoto, N., et al., *Cardiovascular Effects of 1 Year of Progressive and Vigorous Exercise Training in Previously Sedentary Individuals Older Than 65 Years of Age*. Circulation, 2010. **122**(18): p. 1797-1805.
20. Turner, M.J., et al., *Effect of Endurance Exercise Training on Left Ventricular Size and Remodeling in Older Adults With Hypertension*. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2000. **55**(4): p. M245-M251.
21. Lalande, S., R.J. Petrella, and J.K. Shoemaker, *Effect of exercise training on diastolic function in metabolic syndrome*. Applied Physiology, Nutrition, and Metabolism, 2012. **38**(5): p. 545-550.
22. Lakatta, E.G. and D. Levy, *Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease*. Circulation, 2003. **107**(2): p. 346-54.
23. Lam, C.S., et al., *Cardiac structure and ventricular-vascular function in persons with heart failure and preserved ejection fraction from Olmsted County, Minnesota*. Circulation, 2007. **115**(15): p. 1982-90.

24. Borlaug, B.A., et al., *Global Cardiovascular Reserve Dysfunction in Heart Failure With Preserved Ejection Fraction*. Journal of the American College of Cardiology, 2010. **56**(11): p. 845-854.
25. Hartley, L.H., et al., *Physical Training in Sedentary Middle-aged and Older Men III. Cardiac Output and Gas Exchange at Submaximal and Maximal Exercise*. Scandinavian Journal of Clinical & Laboratory Investigation, 1969. **24**(4): p. 335-344.
26. Seals, D.R., et al., *Endurance training in older men and women. I. Cardiovascular responses to exercise*. Journal of Applied Physiology, 1984. **57**(4): p. 1024-1029.
27. Beere, P.A., et al., *Aerobic Exercise Training Can Reverse Age-Related Peripheral Circulatory Changes in Healthy Older Men*. Circulation, 1999. **100**(10): p. 1085-1094.
28. Schulman, S.P., et al., *Continuum of Cardiovascular Performance Across a Broad Range of Fitness Levels in Healthy Older Men*. Circulation, 1996. **94**(3): p. 359-367.
29. Aronson, D., *Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes*. J Hypertens, 2003. **21**(1): p. 3-12.
30. Fahs, C.A., et al., *Resting and post exercise arterial-ventricular coupling in endurance-trained men and women*. J Hum Hypertens, 2013. **27**(9): p. 552-6.

31. Chen, C.-H., et al., *Verapamil acutely reduces ventricular-vascular stiffening and improves aerobic exercise performance in elderly individuals*. Journal of the American College of Cardiology, 1999. **33**(6): p. 1602-1609.
32. Tjonna, A.E., et al., *Aerobic Interval Training Versus Continuous Moderate Exercise as a Treatment for the Metabolic Syndrome: A Pilot Study*. Circulation, 2008. **118**(4): p. 346-354.
33. van Tienen, F.H.J., et al., *Physical Activity Is the Key Determinant of Skeletal Muscle Mitochondrial Function in Type 2 Diabetes*. Journal of Clinical Endocrinology & Metabolism, 2012. **97**(9): p. 3261-3269.
34. McGuire, D.K., et al., *A 30-Year Follow-Up of the Dallas Bed Rest and Training Study*. Circulation, 2001. **104**(12): p. 1350-1357.
35. Stratton, J.R., et al., *Differences in cardiovascular responses to isoproterenol in relation to age and exercise training in healthy men*. Circulation, 1992. **86**(2): p. 504-12.
36. Wisloff, U., et al., *Increased contractility and calcium sensitivity in cardiac myocytes isolated from endurance trained rats*. Cardiovasc Res, 2001. **50**(3): p. 495-508.
37. Vaitkevicius, P.V., et al., *Effects of age and aerobic capacity on arterial stiffness in healthy adults*. Circulation, 1993. **88**(4 Pt 1): p. 1456-62.
38. McTiernan, A., et al., *Exercise effect on weight and body fat in men and women*. Obesity (Silver Spring), 2007. **15**(6): p. 1496-512.
39. Houmard, J.A., et al., *Effect of the volume and intensity of exercise training on insulin sensitivity*. J Appl Physiol, 2004. **96**(1): p. 101-6.

40. Blair, S.N., et al., *Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men.* JAMA, 1995. **273**(14): p. 1093-8.
41. Blair, S.N. and S. Brodney, *Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues.* Med Sci Sports Exerc, 1999. **31**(11 Suppl): p. S646-62.
42. Gottdiener, J.S., et al., *American Society of Echocardiography recommendations for use of echocardiography in clinical trials.* J Am Soc Echocardiogr, 2004. **17**(10): p. 1086-119.
43. Tischler, M.D. and J.F. Plehn, *Applications of stress echocardiography: beyond coronary disease.* J Am Soc Echocardiogr, 1995. **8**(2): p. 185-97.
44. Tartièrre-Kesri, L., et al., *Increased Proximal Arterial Stiffness and Cardiac Response With Moderate Exercise in Patients With Heart Failure and Preserved Ejection Fraction.* Journal of the American College of Cardiology, 2012. **59**(5): p. 455-461.
45. Bhella, P.S., et al., *Abnormal haemodynamic response to exercise in heart failure with preserved ejection fraction.* Eur J Heart Fail, 2011. **13**(12): p. 1296-304.
46. Haykowsky, M.J., et al., *Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction.* J Am Coll Cardiol, 2011. **58**(3): p. 265-74.

Table 1. Effects of exercise training on body composition and metabolic biomarkers in

MetS

	MetS non-trained (n=10)				MetS trained (n=10)			
	Pre		Post		Pre		Post	
Age, years	43 ± 3				48 ± 3			
Sex, female %	60				70			
Height, cm	171 ± 3				168 ± 3			
Weight, kg	99	± 7	99	± 7	105	± 6	105	± 6
Lean Mass, kg	64	± 4	64	± 4	59	± 5	59	± 2
Body fat, %	35	± 2	35	± 3	43	± 3†	43	± 2†
BSA, m ²	2.10	± 0.08	2.10	± 0.09	2.13	± 0.07	2.12	± 0.07
BMI, kg/m ²	34	± 2	34	± 2	37	± 2	37	± 2
Waist circumference, cm	104	± 3	103	± 5	124	± 10	122	± 10
Triglycerides, mg/dL	149	± 21	169	± 20	116	± 17	140	± 24
HDL, mg/dL	41	± 4	40	± 2	47	± 5	45	± 4
Glucose, mg/dL	98	± 2	97	± 3	99	± 3	97	± 3
HbA1c, %	5.7	± 0.1	5.5	± 0.1	5.7	± 0.1	5.6	± 0.1
Insulin, µIU/mL	10.4	± 2.4	10.7	± 2.2	9.0	± 1.5	9.0	± 1.8
HOMA-IR	1.34	± 0.30	1.27	± 0.30	1.23	± 0.19	1.45	± 0.34
Hypertensive (>140/90) %	70				60			
Diabetes Mellitus %	0				0			
Medications, %								
Hypertension	20				30			
Cholesterol	0				10			

Values are mean ± sem; BSA: body surface area; BMI: body mass index; HDL: high density lipoprotein; HbA1c: hemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance.

†p<0.05 vs, MetS non-trained group at specific visit (i.e., Pre or Post)

Of note no significant differences were found compare pre and post values within a group

Table 2: Effects of exercise training on supine left ventricular geometry and diastolic function in MetS

	MetS non-trained (n=10)				MetS trained (n=10)			
	Pre		Post		Pre		Post	
LV geometry								
Septal wall thickness, cm	0.95	± 0.05	0.96	± 0.06	0.92	± 0.06	0.92	± 0.05
Posterior wall thickness, cm	0.87	± 0.06	0.89	± 0.06	0.86	± 0.07	0.93	± 0.06
LV internal dimension, cm	4.41	± 0.13	4.38	± 0.16	4.73	± 0.16	4.67	± 0.12
LV Mass, g	161	± 8	164	± 11	181	± 21	187	± 16
LV Mass Index, g/m ²	77	± 3	78	± 3	84	± 9	88	± 6
Relative Wall Thickness	0.40	± 0.03	0.42	± 0.04	0.36	± 0.03	0.40	± 0.02
LV diastolic function								
E, m/s	0.75	± 0.03	0.75	± 0.04	0.88	± 0.05	0.92	± 0.06
A, m/s	0.64	± 0.03	0.60	± 0.04	0.77	± 0.07	0.74	± 0.07
E/A ratio	1.21	± 0.07	1.29	± 0.07	1.24	± 0.14	1.32	± 0.13
IVRT, m/s	76	± 5	71	± 5	75	± 8	61	± 5
Dec T, m/s	203	± 12	204	± 14	193	± 9	175	± 8
e', m/s	0.12	± 0.01	0.13	± 0.01	0.11	± 0.01	0.11	± 0.01
E/e'	6.20	± 0.26	6.21	± 0.46	9.28	± 1.05	9.13	± 1.18
LV EDP, mmHg	16	± 1	16	± 1	18	± 1	17	± 1

Values are mean ± SEM; E: peak velocity of the early diastolic mitral flow; A: peak velocity of the late diastolic mitral flow; E/A: E divided by A; IVRT: isovolumetric relaxation time; Dec T: mitral flow deceleration time of early filling velocity; e': mitral annular early diastolic velocity; E/e': E divided by e'; LV EDP: left ventricular end-diastolic pressure. Of note no significant differences were found compare pre and post values within a group

Table 3. Effects of exercise training on cardiovascular function in MetS

	MetS non-trained (n=10)					MetS trained (n=10)				
	Pre		Post			Pre		Post		
EDVi, ml/m ² Seated	47	± 2	53	± 3		47	± 2	49	± 2	
Peak ESVi, ml/m ² Seated	49	± 2	51	± 2		46	± 2	52	± 3	
	21	± 1	23	± 2		20	± 1	20	± 1	
Peak SVi, ml/m ² Seated	18	± 1	17	± 1		17	± 2	14	± 2*	
	26	± 2	30	± 2		26	± 2	29	± 1	
Peak HR, bpm Seated	31	± 2	35	± 2		29	± 2	38	± 2*	
	68	± 4	67	± 4		65	± 3	64	± 3	
Peak CI, L·min/m ² Seated	164	± 4	157	± 4*		154	± 6	151	± 6	
	1.81	± 0.16	2.05	± 0.15		1.70	± 0.11	1.85	± 0.12	
Peak EF, % Seated	4.98	± 0.27	5.39	± 0.32		4.50	± 0.42	5.78	± 0.46*	
	56	± 2	58	± 2		56	± 2	60	± 1	
Peak [#] SBP, mmHg Seated	62	± 2	67	± 3		63	± 3	74	± 2*†	
	127	± 4	122	± 3		121	± 4	122	± 3	
Peak DBP, mmHg Seated	192	± 6	190	± 6		188	± 5	185	± 7	
	82	± 3	80	± 3		83	± 2	80	± 1	
Peak ESP, mmHg Seated	73	± 8	76	± 4		76	± 5	64	± 7	
	117	± 4	110	± 2		109	± 4	110	± 3	
Peak SVRi, dyne·m ² /s·cm ⁻⁵ Seated	173	± 5	171	± 5		169	± 4	167	± 6	
	4593	± 381	3848	± 256*		4729	± 360	4245	± 301	
Peak [#]	1877	± 141	1755	± 115		2243	± 244	1565	± 167*	

Values are mean ± SEM; EDVi: end-diastolic volume index; SVi: stroke volume index; ESVi: end-systolic volume index; Ci: cardiac output index; EF: ejection fraction; SBP: systolic blood pressure; DBP: diastolic blood pressure; ESP: end systolic blood pressure; SVRi: systemic vascular resistance index. *p≤0.05 compared to Pre-values within a group (MetS-NonT or MetS-ExT); [‡]significant (p≤0.05) group (MetS-NonT vs. MetS-ExT) by time (pre to post) interaction. †p<0.05 vs, MetS non-trained group at specific visit (i.e., Pre or Post)

Table 4: Effects of exercise training on aerobic capacity in MetS

	MetS non-trained (n=10)				MetS trained (n=10)			
	Pre		Post		Pre		Post	
Ventilatory threshold, l/min	1.13	± 0.14	1.27	± 0.12	1.20	± 0.13	1.56	± 0.21*
Ve/VCO ₂ slope	34.8	± 1.49	34.4	± 1.27	34.4	± 1.4	35.7	± 1.2*
Respiratory exchange ratio	1.13	± 0.03	1.10	± 0.01	1.10	± 0.02	1.09	± 0.02
BORG scale	19	± 0.3	19	± 0.2	19	± 0.5	19	± 0.4
Peak VO ₂ , (L/min) [‡]	1.89	± 0.20	1.84	± 0.18	1.68	± 0.16	2.00	± 0.19*
Peak VO ₂ , (ml/kg/min) LM [‡]	29.4	± 2.2	28.7	± 1.5	29.4	± 1.6	35.0	± 1.7*†
Peak VO ₂ , (ml/kg/min) BW [‡]	19.1	± 1.6	18.6	± 1.2	16.2	± 1.0	19.4	± 1.0*†
Peak A-VO ₂ Diff, ml/100ml	18.4	± 2.0	16.4	± 1.2	18.5	± 1.5	16.2	± 1.0

Values are mean ± SEM; A-VO₂ Diff: arteriovenous oxygen difference. *p≤0.05 compared to Pre-values within a group (MetS-NonT or MetS-ExT); [‡]significant (p≤0.05) group (MetS-NonT vs. MetS-ExT) by time (pre to post) interaction. †p<0.05 vs, MetS non-trained group at specific visit (i.e., Pre or Post)

Figure Legends

Figure 1. Change in arterial-ventricular coupling (Ea/Ees), LV end-systolic elastance (Ees), and arterial elastance (Ea) from rest to peak exercise in Mets who underwent exercise training (MetS-ExT, diamond) and in MetS who remained inactive (MetS-NonT, triangles). Post intervention for both MetS group is depicted by a dashed line. Exercise training significantly reduced peak Ea/Ees, and increased peak Ees in MetS, and there was a significant time (pre and post intervention) by group (MetS-ExT vs. MetS-NonT) for Ea/Ees and Ees. * $p < 0.05$ illustrates significant differences pre and post intervention in MetS Ex-T; + $p < 0.05$ time by group interaction. Data presented as means $\pm$ SEM.

Figure 1.

Chapter 5

ACUTE RESVERATROL SUPPLEMENTATION IN PEOPLE WITH METABOLIC SYNDROME: A RANDOMIZED, PLACEBO-CONTROLLED STUDY OF ARTERIAL STIFFNESS AND CENTRAL BLOOD PRESSURE

Sara B. Fournier^{1, 2}, Evan DeVallance^{1, 2}, David A. Donley¹, Daniel E. Bonner¹, Stephen A. Alway¹, and Paul D. Chantler^{1, 2}

¹Division of Exercise Physiology, School of Medicine, West Virginia University; ²Center for Cardiovascular and Respiratory Sciences, School of Medicine, West Virginia University

Running Head: Resveratrol and Metabolic Syndrome

Abstract

Background and Aims: Large elastic artery stiffening (AS) is a feature of normative aging and reflects a process by which degenerative adaptations in large arteries lead to wall stiffening. Metabolic syndrome (MetS) is associated with pathological vascular adaptations including AS which may play a role in mediating CVD risk in this population. Resveratrol (RSV), a naturally occurring polyphenol, has been shown to indirectly increase bioavailability of nitric oxide through changes in vascular oxidative stress and inflammation. Thus, RSV may represent an ideal therapy to target AS in MetS. The objective of this study was to investigate whether single oral doses of RSV can reduce AS and central aortic blood pressure in large elastic arteries of individuals with MetS.

Methods and Results: 28 subjects (28-70 years): 17 healthy controls and 11 MetS consumed two doses of RSV (500 and 1000 mg) and a placebo at weekly intervals in a double-blind, randomized fashion. AS and BP were measured before and at 45 and 75 minutes after consumption of the supplement. Plasma samples were obtained at baseline and at 60 minutes after consumption of the supplement and subsequently analyzed for RSV concentration. Data were analyzed by a two-way repeated-measures analysis of variance. No significant effect of RSV was observed, however, detectable concentration of RSV and its primary metabolites was shown in a small subset of participants (n=20).

Conclusion: Acute supplementation with 500 or 1000mg of RSV had no observable effect on AS or blood pressure at 45 or 75 minutes post-consumption. These results warrant further investigation of the use of supplementation with RSV as a strategy to target AS in MetS.

Introduction

Large elastic artery stiffening (AS) occurs as a result of normative biological aging, and describes a process by which degenerative adaptations in the conduit arteries lead to wall stiffening. In the presence of AS, the heart must fulfill the increased force requirements to accommodate the less compliant arteries. Over time, the additional load placed on the heart leads to LV hypertrophy (LVH) and heart failure. Importantly, AS has notable functional implications on the large elastic arteries, as it contributes to a diminishing of the Windkessel effect, or in other words the ability of the elastic aorta to maintain a continuous and steady blood flow to peripheral vessels [1]. AS is increased in the presence of CVD and results in elevations in pulse pressure at the site of central and peripheral arteries, which can extend to the microcirculation contributing to target organ damage [2-5].

The metabolic syndrome (MetS) is defined by the co-occurrence of clinical criteria and is used to identify patients at high risk for CVD, type II diabetes (T2DM), and all cause mortality and accordingly, has become an important therapeutic target of lifestyle modification. MetS is associated with pathological vascular adaptations including, but not limited to, AS [6, 7]. AS is known to be significantly increased in MetS compared to age-matched healthy controls [6, 8]. In MetS, AS may have a detrimental impact on cardiac function; Increases in central systolic blood pressure that accompany AS, promote cardiac hypertrophy leading to decreases in diastolic pressure, and impairment of coronary perfusion [9]. Thus, the increased risk of CVD associated with MetS may be due, in part, to increased AS. Indeed, data from our laboratory demonstrates that MetS patients have increased AS, arterial wall remodeling, abnormal

resting cardiac diastolic function, impaired peak exercise cardiac contraction, and reduced aerobic capacity compared to non-MetS [6]. The increase in AS observed in MetS is of clinical importance as a 1.0 m/second increase in AS is associated with a 15-18 percent increase risk of CV events [10, 11]. Promising evidence from animal [12-14] and initial human research [15, 16] demonstrate the potential of resveratrol (RSV) for CV protection. Specifically, the cardioprotective effects of RSV supplementation have been attributed to the actions of RSV to acutely reduce inflammation and oxidative stress; two phenomena associated with MetS that are known to alter the arterial wall, decrease the bioavailability of the vasodilator nitric oxide (NO) and contribute to acute changes in AS [17-20]. Furthermore, RSV has been demonstrated to exert a vasorelaxing effect in an ex-vivo preparation of abdominal aortic rings of Sprague-Dawley rats [21]. The vasorelaxing effects of RSV in Sprague-Dawley aortic rings were attributed to endothelial-dependent and endothelial-independent mechanisms, opening of the K⁺ channel resulting in hyperpolarization of vascular smooth muscle, and inhibition of extracellular calcium influx and intracellular calcium release in smooth muscle cells [21].

While changes in structural components of the arterial wall occur over a longer duration to negatively increase AS, acute changes may also occur to mediate stiffness. Notably, changes in vascular smooth muscle cell (VSMC) tone and sympathetic nervous system activity have been shown to acutely modulate AS [22]. More specifically, sympathetic stimulation can alter AS through an indirect change in arterial pressure or through a direct increase in vascular tone, causing vasoconstriction and decreased arterial diameter [23-25]. Moreover, impaired endothelial dysfunction, as a

result of low-grade inflammation [26] enhances vasoconstrictor activity, altering large artery smooth muscle tone [27-29] which may contribute to acute changes in AS.

Accordingly, nutritional supplementation with RSV may represent a promising therapy for reducing AS, and subsequently alleviating CVD risk in individuals with MetS. Therefore, the objective of the current randomized, double-blind, placebo-controlled study was to investigate whether single oral doses of RSV can reduce AS and central aortic blood pressure (cSBP) in large elastic arteries of individuals with MetS. We hypothesized that treatment with single doses of RSV would result in acute reductions in AS and cSBP in individuals afflicted with MetS.

Materials

Study Population

The study population consisted of 28 subjects (28-70 years): 17 healthy controls (44 ± 3 ; 83% Female) and 11 MetS (51 ± 3 ; 64% Female). Healthy controls were free from clinically manifest CVD as determined by a detailed medical history, physical examination, and evidence of normal resting and exercise electrocardiograms. MetS subjects were free from clinically manifest CVD and diabetes mellitus. None of the participants were current or former smokers or were currently receiving treatment for peripheral artery disease. Exclusion criteria included T2DM (HbA1c $\geq 6.5\%$ or use of diabetic medications), pulmonary disease, angina, atrial fibrillation, aortic stenosis, anemia, myocardial infarction, stroke, or coronary revascularization as assessed by a detailed medical history, physical examination, and a resting and exercise electrocardiogram. Subjects who participated in regular exercise, defined as >30 min, 3 times/week were excluded. All participants signed a written informed consent form that was approved by the Institutional Review Board of West Virginia University.

Definition of the Metabolic Syndrome

MetS was defined according to the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP ATPIII) [30] which requires three or more of the following five components: abdominal obesity (waist men >102 cm, women >88 cm), low HDL cholesterol (men <40 mg/dl; women <50 mg/dl), hypertriglyceridemia (≥ 150

mg/dl), elevated fasting glucose (≥ 100 mg/dl and < 126 mg/dl) and elevated blood pressure ($\geq 130/\geq 85$ mmHg or use of hypertensive medications).

Study Design

All assessments were performed between 7:00 and 10:00AM in a quiet, temperature-controlled room following a 12-hour fast and abstinence from alcohol, caffeine, and vitamins. Participants were asked to withhold all cardiovascular medications for at least 24 hours prior to assessments. All participants were asked to maintain current dietary habits and to limit excessive polyphenol intake (including consumption of peanuts, red wine, green tea, etc.) for the duration of the study.

Participants attended the laboratory on 4 separate occasions. The first occasion was for the completion of a screening visit to acquire patient consent and to perform anthropometric measures. On each of the 3 remaining visits, participants consumed 2 identical capsules containing the following: inert placebo or 500mg *trans*-resveratrol. The capsules were combined to achieve combinations of placebo, 500mg of *trans*-resveratrol, or 1000mg of *trans*-resveratrol. Each treatment was assigned an A, B, or C by an independent staff member. Each treatment was consumed *ad libitum* with water at weekly intervals in a double blind randomized fashion. No member of the investigational team was aware of the contents of the capsules until a blind-data review was completed.

On completion of anthropometric measurements and a minimum of 15 minutes of quiet supine rest supine measures of baseline arterial structure and function were performed followed by a baseline venous blood draw. Based on pharmacokinetic

results, peak RSV concentrations occur between 0.8-1.5 hours following administration of RSV [31]. Thus, measures of arterial structure and function were repeated at 45 and 75 minutes post-capsule consumption. An additional venous blood draw was collected at 60 minutes post-capsule consumption.

Anthropometric Measurements

At screening visit, height, weight, and waist and hip circumferences were determined using standard laboratory procedures for all participants. Waist circumference was assessed at the site of the smallest circumference between the rib cage and the iliac crest with subjects in a standing position. Hip circumference was assessed at the site of the largest circumference between the waist and thighs. Lean body mass and fat mass were measured using air displacement plethysmography (BodPod®; Life measurement, Concord, CA, USA). During the assessment of body composition subjects were dressed in close-fitting bathing suits and a swim cap. Body mass index (BMI) was calculated as weight (kg) / height (m)².

Arterial Function

Brachial systolic (SBP) and diastolic (DBP) blood pressures were measured using an automated, oscillometric sphygmomanometer (Critikon Dinamap Compact BP monitor, GE Medical, Tampa, Fla, USA) and pulse pressure (PP) was calculated from SBP-DBP. Pulse wave analysis was performed noninvasively on the radial artery (SphygmoCor system, AtCor Medical, Sydney, Australia). All measurements were made in triplicate, and the mean values used for subsequent analysis. The SphygmoCor

system synthesizes a central (ascending aortic) pressure waveform from the radial pressure waveform that does not differ from that of an intra-arterially recorded wave [32] using a validated generalized transfer function [33]. These waveforms were calibrated against brachial mean arterial and diastolic pressure to estimate aortic pressures. The characteristics of the aortic pulse wave (Figure 1) were determined as previously described [34] using established guidelines [35].

Carotid to femoral pulse wave velocity (cfPWV; central arterial stiffness) and carotid to radial pulse wave velocity (crPWV; peripheral arterial stiffness) were measured by applanation tonometry (AtCor Medical, Sydney, Australia). ECG-gated waveforms were sequentially recorded. Aortic distance (D) was calculated as the difference in the distances from the carotid to the suprasternal notch and from the suprasternal notch to the femoral artery or radial artery. Time delay was calculated using a foot-of-the-wave method.

Blood Collection and Analysis

Venous blood sampling was performed between 7:00 and 10:00AM following an overnight 12-hour fast at baseline and again at 60 minutes following capsule consumption. Patient plasma acquired from venous blood samples was sent to West Virginia University Hospital's central laboratory in Morgantown, WV for lipid profile analysis. Total cholesterol, HDL cholesterol, triglycerides, and fasting blood glucose were determined in plasma (lithium heparin, Becton Dickinson plasma separator tubes) using Beckman Coulter (Brea, CA) DxC automated chemistry analyzers. Total cholesterol was measured using a cholesterol esterase/cholesterol oxidase-or

peroxidase-driven, timed endpoint method (coefficient of variation, <7%). Triglycerides were measured using a lipase/glycerol kinase/glycerophosphate oxidase/horseradish peroxidase-driven, timed endpoint method (coefficient of variation, 5%-7%). Glucose was measured electrochemically using a glucose oxidase-/catalase-/molybdate-driven oxygen rate method (coefficient of variation, <5%). Glycated hemoglobin (A1c fraction) was measured in whole blood (K₂-EDTA; Becton Dickinson) using a Bio-Rad (Hercules, CA) Variant II Turbo high-performance liquid chromatography system (coefficient of variation, <2%). Insulin was measured in serum (untreated/red top tube; Becton Dickinson) on an Immulite 2000 immunochemistry system (Siemens) (coefficient of variation, <10%).

Plasma Concentration of Resveratrol and its Metabolites

Select plasma samples (n=20) were delivered to Protea Biosciences, Inc. (protea®, Morgantown, WV) for determination of resveratrol and its primary metabolites. Plasma concentrations of resveratrol (3,5,4'-trihydroxy-trans- stilbene) and its primary metabolites, including trans-resveratrol-3-O-sulfate, trans-resveratrol-3-O-glucuronide, and trans-resveratrol-4-O-glucuronide were determined using liquid chromatography tandem mass spectrometry (HPLC: Shimadzu LC-20AD HPLC, Tokyo, Japan); MS: Triple-Quad 4000, ABSciex Toronto, Canada). Briefly, fasting venous blood samples (15-20ml) were drawn at baseline and at 60 minutes post-capsule ingestion of 500mg of *trans*-resveratrol, 1000mg of *trans*-resveratrol or placebo in a small sample of participants (n=20) in EDTA coated tubes. Blood samples were processed into plasma and stored at -80°C before extraction. Plasma samples were delivered to Protea

Biosciences, Inc. (protea®, Morgantown, WV) for the determination of concentrations of resveratrol and its metabolites.

Calibration and quality control solutions were prepared by spiking human plasma with solutions of varying concentrations of resveratrol and metabolites in 50:50 methanol:water. Blank solutions were prepared by spiking human plasma with 50:50 methanol:water. All standards, controls, and blanks were then aliquotted into 1.5µl microcentrifuge tubes using a 50µl sample volume.

All plasma samples were thawed on the bench top at room temperature, vortexed for 1 minute and aliquoted into 1.5µl microcentrifuge tubes using a 50µl sample volume. Acetonitrile was added to all internal standards. Next, 200µl of Working Internal Standard Solution (50ng/ml resveratrol- $^{13}\text{C}_6$ in acetonitrile) was added to all other samples using a repeater pipet to precipitate the proteins. All tubes were capped and vortexed for 1 minute. The samples were then centrifuged for 5 minutes at 10,000 RMP. 100µl of water was added to each sample well of a Nunc (U96 PP 2ml) 96-well plate. The plate was then sealed with a pre-slit capmat, vortexed for 1 minute, and stored at 4°C.

Statistical Analysis

Normality was evaluated by the Kolmogorov-Smirnov test. Categorical variables were compared by the chi-square test, whereas baseline comparisons between groups were analyzed by an independent t-test or Mann–Whitney test. The changes from baseline to 45 and 75 minutes post capsule consumption in arterial parameters were examined by a time (baseline to 45 or 75 minutes post capsule consumption) by group

(MetS vs. Controls) by treatment (placebo, 500mg RSV, or 1000mg RSV) interaction evaluated using a two-way repeated-measures analysis of variance with Tukey's post hoc test. Additionally, the changes from baseline to 45 and 75 minutes post capsule consumption in arterial parameters were examined by a time (baseline to 45 or 75 minutes post capsule consumption) by treatment (placebo, 500mg RSV, or 1000mg RSV) interaction evaluated using a two-way repeated-measures analysis of variance with Tukey's post hoc test. Multiple linear regression analysis was used to determine whether age, risk score, and baseline measurements could predict changes in arterial and central blood pressure parameters following supplementation with RSV. Data are presented as means $\pm$ s.e.m. and $P < 0.05$ was required for significance. All analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA).

Results

Subject Characteristics

Baseline characteristics of the study cohort are presented in Table 1. Twenty-eight participants were recruited from the surrounding Morgantown area and were separated into two groups, healthy controls and MetS, based on the NCEP ATPIII guidelines for the definition of MetS. Age and sex were similar between groups despite an overall majority of female participants. As expected, MetS participants had significantly elevated baseline CVD risk factors.

Baseline Arterial Measurements

Baseline arterial measurements for MetS and controls are represented in Table 2. MetS and controls exhibited similar baseline values in arterial parameters with the exception of brachial and central systolic blood pressure, SEVR, and AS as measured using cfPWV. In agreement with previous findings from our laboratory [6, 34, 36] MetS were characterized by general increases in bSBP and cSBP and increased AS compared to healthy controls.

Resveratrol Plasma Concentrations

Mean plasma concentrations of *trans*-resveratrol and its conjugates at baseline and 60 minutes post-capsule consumption in a small sub sample of participants (n=20) are shown in Figure 2. Due to rapid and extensive metabolism and formation of metabolites including resveratrol glucuronides and resveratrol sulfates. Unmetabolized *trans*-resveratrol reached small but detectable concentrations in plasma following oral

doses of 500 mg and 1000 mg. These results demonstrate that, irrespective of the presence or absence of MetS, plasma RSV and three of its primary metabolites were detectable at 60 minutes post-oral supplementation of 500mg or 1000mg of RSV. Our resolution to examine RSV at doses of 500mg/day and 1000mg/day was primarily based on the understanding that RSV when administered orally is well absorbed but exhibits a very low bioavailability, and has been shown to be well tolerated in humans with no marked toxicity reported at much higher dosages as compared to our selected dosage. Evidence indicates that a range of dosages from 30mg to 5000mg produce various physiological effects in humans [37-40]. These results demonstrate the feasibility of administering RSV at levels detectable in plasma.

Relationship between Resveratrol Supplementation on AS and Blood Pressure

Graphical representation of the change in arterial stiffness and central blood pressure parameters at 45 and 75 minutes post oral supplementation with placebo, 500mg RSV, and 1000 mg RSV is shown in Figures 3-5. Using a two-way we evaluated the effect of RSV to elicit changes in Arterial and central blood pressure parameters at 45 and 75 minutes post capsule consumption by a time (baseline to 45 or 75 minutes post capsule consumption) by group (MetS vs. Controls) by treatment (placebo, 500mg RSV, or 1000mg RSV) interaction evaluated using a two-way repeated-measures analysis of variance. Interestingly, no significant effect of resveratrol on arterial and central blood pressure parameters was identified between MetS and healthy controls. Next, we repeated this statistical analysis without separation of participants into MetS and control groups. Similarly, no time (baseline to 45 or 75 minutes post capsule

consumption) by treatment (placebo, 500mg RSV, or 1000mg RSV) effects were observed.

Moving forward, we wanted to understand whether changes in AS and blood pressure following supplementation with RSV can be predicted based on age, riskpoints, and baseline values of AS and blood pressure. Using multiple regression analysis we report that baseline bPP and cPP significantly predict changes in bPP and cPP 75 respectively, 75 minutes following oral ingestion of 500mg of RSV. Additionally, baseline values of bSBP, bPP, and cPP significantly predict changes in bSBP, bPP, and cPP, respectively, 45 minutes following oral ingestion of 1000mg of RSV. Importantly the these relationships were inverse, meaning that higher baseline values of select brachial and central blood pressure measurements predict decreases in delta values (baseline – 45 or 75 minutes) following treatment with RSV. Results from multiple regression analysis are depicted in Table 3.

Discussion

AS occurs with advancing age and is further elevated in MetS compared to age-matched healthy controls [6, 8]. Complications of AS include the development of LVH, reductions in coronary perfusion [9]. Thus, the increased risk of CVD associated with MetS may be due, in part, to increased AS. Indeed, data from our laboratory demonstrates that MetS patients have increased AS, arterial wall remodeling, abnormal resting cardiac diastolic function, impaired peak exercise cardiac contraction, and reduced aerobic capacity compared to non-MetS [6]. Importantly, changes in vascular smooth muscle cell (VSMC) tone and sympathetic nervous system activity have been shown to acutely modulate AS [22] and may represent effective therapeutic targets for affecting improvements in AS and likely CVD in MetS.

Resveratrol, is a polyphenolic compound found in dietary sources including, but not limited to, grape skins, peanuts, and red wine. In the last decade RSV has generated substantial interest among the scientific community and within popular culture for its CV protective potential. Namely, RSV has been shown to reduce inflammation and oxidative stress, as well as to promote smooth muscle vasorelaxation through both chronic and acute mechanisms that remain unclear. For these reasons, we sought to investigate the effects of two single doses of RSV on arterial and blood pressure parameters in MetS and healthy controls.

A comparison between MetS and controls to determine the whether RSV was able to improve AS and blood pressure showed no significant interactions. These findings precluded our original hypothesis that 500mg or 1000mg of RSV would affect acute improvements in AS and blood pressure, and further, that we would be able to

capture these acute improvements at 45 and 75 minutes following capsule ingestion in MetS. Further investigation revealed the ability of some baseline blood pressure parameters to predict how they would change in response to treatment with RSV. While the literature regarding RSV treatment in clinical research is still in its infancy and is largely controversial, the results from this study are in contrast to recent evidence that RSV at higher doses (≥ 150 mg/day) significantly decreases SBP [41]. Further, single doses of RSV have recently been demonstrated to acutely improve flow-mediated dilation (FMD) in a dose-dependent manner [39].

Although the data presented here do not find a significant short-term effect of 500 and 1000mg of RSV on AS and central blood pressure, it must be emphasized that our results should neither discourage nor slight the actions of RSV as a therapeutic approach to human disease. It is true that we are unable to draw a significant positive conclusion for our investigation of whether 500mg and 1000mg can acutely alter AS and blood pressure in MetS; however, by the same token we are unable to demonstrate that RSV exerts any negative effects that may exacerbate AS, or contribute to elevated blood pressure in MetS. Moreover, addressing the many limitations and caveats of this study may shed light on the inconclusive nature of our current findings.

Limitations

First, due to the small sample size of our MetS and control groups our study is underpowered for the comparisons required to determine a time by treatment by group effect on AS. For this reason these data should be regarded as preliminary until we can

obtain data on a larger population sample. While we reported non-significant results from multivariate modeling, we can speculate that in MetS significant elevations in baseline parameters of AS and blood pressure, may indicate a greater potential for improvement in these parameters and warrants further investigation. Second, although age and sex were similar between groups in our study, we included a wide range of aged individuals, a majority of which were women, begging the question as to whether there may be divergent mechanisms by which RSV can exert its effects in men and women and whether or not these differences have the potential to mask improvements in AS conferred by RSV require further investigation in a cohort with more equal numbers of both men and women. Third, we did not provide a measure of autonomic function in this study. The potential affects of RSV on autonomic function [42], which has been shown to modulate acute changes in AS [22], was not assessed, but future studies should consider this mechanism which can be easily accomplished in the short-term by recording beat-to-beat changes in BP and HR. Additional concerns that may limit the interpretation of this study include patient selection. While comparisons of group demographics and baseline parameters of AS and blood pressure were indicative of our ability to successfully screen for MetS, the elevations in this small group were only moderate, especially with respect to age and elevations in blood lipid profile, blood pressure, and AS. As MetS can be considered to exist as a spectrum in terms of disease severity, the participants recruited in this study may represent less severe MetS. It is possible that the determination of the therapeutic potential of RSV to reduce AS may require the use of participants with more severe disease. Of equal importance is our resolution to examine two single doses of RSV. Future studies to evaluate the

therapeutic potential of RSV may be more clinically relevant if they are designed with the goal of demonstrating long-term disease prevention and maintenance of CV health via chronic supplementation with RSV. With that in mind, the acute nature of our study may be too short to show the benefits of RSV for lowering CVD risk in MetS. Finally, our resolution to examine two time points based on pharmacokinetic data in the existing literature may have inherently limited our resolution for detecting improvements in AS and blood pressure. Data from plasma concentrations of RSV and its primary metabolites suggest that delivery of RSV is not a limiting factor in this study, however, it is possible that changes elicited by RSV to alter AS or BP may occur before or after our selected time points and future studies would be better to perform a dose-response study in a separate cohort to more accurately select the best time points for investigation into the short-term affects of RSV.

Conclusion

In conclusion, this study demonstrates that acute supplementation with 500 mg and 1000 mg of RSV failed to modulate improvements in AS and blood pressure at 45 and 75 minutes post-supplementation. Taken together, these findings suggest future investigation of the short-term and chronic benefits of RSV for improving AS and blood pressure, with particular attention to patient selection, dose selection, and study duration.

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Reference List

1. Lewington, S., et al., *Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies*. Lancet, 2002. 360(9349): p. 1903-13.
2. Franklin, S.S., et al., *Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study*. Circulation, 1997. 96(1): p. 308-15.
3. Mitchell, G.F., et al., *Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study*. Hypertension, 2004. 43(6): p. 1239-45.
4. Scuteri, A., et al., *The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study*. Eur Heart J, 2010. 31(5): p. 602-13.
5. Tomiyama, H., et al., *Continuous smoking and progression of arterial stiffening: a prospective study*. J Am Coll Cardiol, 2010. 55(18): p. 1979-87.
6. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. Exp Physiol, 2013.
7. Scuteri, A., et al., *Arterial stiffness and influences of the metabolic syndrome: a cross-countries study*. Atherosclerosis, 2014. 233(2): p. 654-60.
8. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. 43(8): p. 1388-95.
9. Azevedo, A., et al., *Increasing number of components of the metabolic syndrome and cardiac structural and functional abnormalities--cross-sectional study of the general population*. BMC Cardiovasc Disord, 2007. 7: p. 17.

10. Lorenz, M.W., et al., *Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis*. Circulation, 2007. 115(4): p. 459-67.
11. Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis, *Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis*. J Am Coll Cardiol, 2010. 55(13): p. 1318-27.
12. Fiori, J.L., et al., *Resveratrol prevents beta-cell dedifferentiation in nonhuman primates given a high-fat/high-sugar diet*. Diabetes, 2013. 62(10): p. 3500-13.
13. Mattison, J.A., et al., *Resveratrol prevents high fat/sucrose diet-induced central arterial wall inflammation and stiffening in nonhuman primates*. Cell Metab, 2014. 20(1): p. 183-90.
14. Rivera, L., et al., *Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats*. Biochem Pharmacol, 2009. 77(6): p. 1053-63.
15. Carrizzo, A., et al., *Resveratrol improves vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism*. Hypertension, 2013. 62(2): p. 359-66.
16. Wong, R.H.X., et al., *Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure*. Nutrition, Metabolism and Cardiovascular Diseases, 2011. 21(11): p. 851-856.
17. Leikert, J.F., et al., *Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells*. Circulation, 2002. 106(13): p. 1614-7.

18. Olas, B. and B. Wachowicz, *Resveratrol and vitamin C as antioxidants in blood platelets*. Thromb Res, 2002. 106(2): p. 143-8.
19. Sonnett, T.E., et al., *Diabetes mellitus, inflammation, obesity: proposed treatment pathways for current and future therapies*. Ann Pharmacother, 2010. 44(4): p. 701-11.
20. Wallerath, T., et al., *Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase*. Circulation, 2002. 106(13): p. 1652-8.
21. Shen, M., et al., *The vasorelaxing effect of resveratrol on abdominal aorta from rats and its underlying mechanisms*. Vascul Pharmacol, 2013. 58(1-2): p. 64-70.
22. Wilkinson, I.B. and C.M. McEniery, *Arterial stiffness, endothelial function and novel pharmacological approaches*. Clin Exp Pharmacol Physiol, 2004. 31(11): p. 795-9.
23. Boutouyrie, P., et al., *Sympathetic activation decreases medium-sized arterial compliance in humans*. Am J Physiol, 1994. 267(4 Pt 2): p. H1368-76.
24. Joannides, R., et al., *Influence of sympathetic tone on mechanical properties of muscular arteries in humans*. Am J Physiol, 1995. 268(2 Pt 2): p. H794-801.
25. Lydakis, C., et al., *Changes of elastic properties of central arteries during acute static exercise and lower body negative pressure*. Eur J Appl Physiol, 2008. 102(6): p. 633-41.
26. Hingorani, A.D., et al., *Acute systemic inflammation impairs endothelium-dependent dilatation in humans*. Circulation, 2000. 102(9): p. 994-9.

27. McEniery, C.M., et al., *Endothelin-1 regulates arterial pulse wave velocity in vivo*. J Am Coll Cardiol, 2003. 42(11): p. 1975-81.
28. Wilkinson, I.B., et al., *Nitric oxide regulates local arterial distensibility in vivo*. Circulation, 2002. 105(2): p. 213-7.
29. Kinlay, S., et al., *Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo*. Hypertension, 2001. 38(5): p. 1049-53.
30. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report*. Circulation, 2002. 106(25): p. 3143-421.
31. Almeida, L., et al., *Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers*. Mol Nutr Food Res, 2009. 53 Suppl 1: p. S7-15.
32. Chen, C.H., et al., *Validation of carotid artery tonometry as a means of estimating augmentation index of ascending aortic pressure*. Hypertension, 1996. 27(2): p. 168-75.
33. Chen, C.H., et al., *Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function*. Circulation, 1997. 95(7): p. 1827-36.
34. Donley, D.A., et al., *Aerobic Exercise Training Reduces Arterial Stiffness in Metabolic Syndrome*. J Appl Physiol (1985), 2014.

35. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications*. Eur Heart J, 2006. 27(21): p. 2588-605.
36. Fournier, S.B., et al., *Improved Arterial-Ventricular Coupling in Metabolic Syndrome after Exercise Training*. Med Sci Sports Exerc, 2014.
37. Boocock, D.J., et al., *Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent*. Cancer Epidemiol Biomarkers Prev, 2007. 16(6): p. 1246-52.
38. Brasnyo, P., et al., *Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients*. Br J Nutr, 2011. 106(3): p. 383-9.
39. Wong, R.H., et al., *Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure*. Nutr Metab Cardiovasc Dis, 2011. 21(11): p. 851-6.
40. Vislocky, L.M. and M.L. Fernandez, *Biomedical effects of grape products*. Nutr Rev, 2010. 68(11): p. 656-70.
41. Liu, Y., et al., *Effect of resveratrol on blood pressure: a meta-analysis of randomized controlled trials*. Clin Nutr, 2015. 34(1): p. 27-34.
42. Dillenburg, D.R., et al., *Resveratrol and grape juice differentially ameliorate cardiovascular autonomic modulation in L-NAME-treated rats*. Auton Neurosci, 2013. 179(1-2): p. 9-13.
43. Mannello, F., et al., *Differences in both matrix metalloproteinase 9 concentration and zymographic profile between plasma and serum with clot activators are due*

to the presence of amorphous silica or silicate salts in blood collection devices.

Analytical Biochemistry, 2008. 374(1): p. 56-63.

Table 1. Patient Demographics and Characteristics

	Control (n=17)	MetS (n=11)
Age, years	44 ± 3	51 ± 3
Sex, % Female	83	64
Height, cm	163.12 ± 2.71	166.75 ± 2.16
Weight, lbs	*147.45 ± 6.4	219.69 ± 8.25
BSA, m ²	*2.40 ± 0.05	2.90 ± 0.06
BMI, kg/m ²	*25.49 ± 1.45	35.83 ± 1.14
Waist Circumference, cm	*82.33 ± 2.90	110.32 ± 3.84
Triglycerides, mg/dL	*79.33 ± 7.67	151.29 ± 21.63
HDL Cholesterol, mg/dL	*57.00 ± 2.75	42.21 ± 1.34
Glucose, mg/dL	*91.50 ± 2.03	118.71 ± 8.16
HbA1c, %	*5.15 ± 0.07	5.88 ± 0.28
Insulin, µIU/mL	*5.76 ± 0.69	17.36 ± 1.81
Riskpoints	*14.89 ± 1.89	39.07 ± 2.45
Avg HR, bpm	69.40 ± 1.93	73.00 ± 2.48
Avg. bSBP, mmHg	*114.38 ± 2.81	124.33 ± 2.87
Avg. bDBP, mmHg	77.15 ± 1.92	82.01 ± 2.21
Avg. bPP, mmHg	37.23 ± 2.39	42.32 ± 3.81
Avg. cSBP, mmHg	*105.52 ± 3.07	113.40 ± 2.23
Avg. cDBP, mmHg	75.02 ± 2.28	78.52 ± 2.62
Avg. cPP, mmHg	30.51 ± 2.05	34.88 ± 3.60
<p>Values expressed as mean ± SEM. Abbreviations: MetS, metabolic syndrome; BSA, body surface area; BMI, body mass index; HDL, high-density lipoprotein; HR, heart rate; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; bPP, brachial pulse pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure.</p> <p>*p<0.05 vs. MetS</p>		

Table 2. Baseline Parameters in MetS and Controls

	Control (n=17)	MetS (n=11)
HR, bpm	66.21 ± 2.06	71.11 ± 2.59
bSBP, mmHg	*110.29 ± 2.60	123.08 ± 3.43
bpDBP, mmHg	73.29 ± 2.03	76.69 ± 2.24
bPP, mmHg	37.00 ± 1.82	46.38 ± 4.67
cSBP, mmHg	*102.01 ± 2.95	111.26 ± 2.37
cDBP, mmHg	74.13 ± 2.05	77.73 ± 2.25
cPP, mmHg	27.82 ± 1.82	34.41 ± 3.63
AP, mmHg	7.11 ± 1.19	6.94 ± 1.05
AGI	23.42 ± 3.23	19.86 ± 2.41
AGI@HR75, %	19.25 ± 13.53	18.11 ± 2.41
SEVR, %	*158.69 ± 5.20	136.97 ± 8.58
cfPWV, m/s	*6.54 ± 0.24	7.73 ± 0.41
<p>Values expressed as mean ± SEM. Abbreviations: MetS, metabolic syndrome; HR, heart rate; pSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; bPP, brachial pulse pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cPP, central pulse pressure; AP, augmentation pressure; AGI, augmentation index; AGI@HR75, augmentation index corrected for a HR of 75; SEVR, sub-endocardial viability ratio; cfPWV, carotid to femoral pulse wave velocity</p> <p>*p<0.05 vs. MetS</p>		

Table 3. Multiple linear regression models

	<i>B</i>	<i>SE B</i>	β	<i>p</i>
Delta bSBP at 45 min post 500mg RSV				
Riskpoints	0.072	0.107	0.129	0.512
Age	0.207	0.100	0.433	0.050
bSBP	-0.269	0.114	-0.470	0.027
Delta bPP at 45 min post 500mg RSV				
Riskpoints	-0.071	0.107	-0.127	0.511
Age	0.190	0.091	0.393	0.048
bPP	-0.305	0.108	-0.519	0.009
Delta cPP at 45 min post 500mg RSV				
Riskpoints	0.064	0.093	0.149	0.496
Age	0.120	0.071	0.323	0.104
cPP	-0.285	0.108	-0.542	0.015
Delta cPP at 75 min post 1000mg RSV				
Riskpoints	0.150	0.089	0.378	0.107
Age	-0.057	0.064	-0.173	0.382
cPP	-.0199	0.097	-0.442	0.051
Delta bPP at 75 min post 1000mg RSV				
Riskpoints	0.072	0.097	0.147	0.465
Age	-0.051	0.079	-0.124	0.529
bPP	-0.211	0.094	-0.431	0.033
<i>B</i> denotes the variable estimate; <i>SE B</i> denotes the standard error of the variable estimate; β denotes the standardized estimate				

Figure Legends

Figure 1. Example of central pressure waveform. The systolic and diastolic pressures are the peak and trough of the pressure waveform. Augmentation pressure (AP) is the pressure added to the forward wave by the reflected wave ($P_1 - P_2$), whereas augmentation index is the ratio between AP and central pulse pressure ($PP = \text{systolic} - \text{diastolic pressure}$).

Figure 2. Mean ($\pm$ SEM) plasma concentrations of trans-resveratrol (A) and its glucuronidated and sulfated conjugates (B) at baseline and 60 minutes-post ingestion of 500mg or 1000mg of RSV in a small sub cohort of participants ($n=20$) as determined by HPLC.

Figure 3. Mean ($\pm$ SEM) changes (45 or 75 minutes – baseline) in cSBP (A) and cPP (B) at 45 and 75 minutes post-supplementation with placebo, 500mg RSV, and 1000mg RSV in MetS and healthy controls. Positive bars represent increases relative to baseline values and negative bars represent reductions relative to baseline values.

Figure 4. Mean ($\pm$ SEM) changes (45 or 75 minutes – baseline) in AP (A) and SEVR (B) at 45 and 75 minutes post-supplementation with placebo, 500mg RSV, and 1000mg RSV in MetS and healthy controls. Positive bars represent increases relative to baseline values and negative bars represent reductions relative to baseline values.

Figure 5. Mean ($\pm$ SEM) changes (45 or 75 minutes – baseline) in AGI (A) and AGI@HR75 (B) at 45 and 75 minutes post-supplementation with placebo, 500mg RSV, and 1000mg RSV in MetS and healthy controls. Positive bars represent increases relative to baseline values and negative bars represent reductions relative to baseline values.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Chapter 6

General Discussion

General Discussion

The overall purpose of the investigations presented within this dissertation was to define CV dysfunction in the metabolic syndrome and to identify practical therapeutic strategies to improve CV function. Specifically, our goals were to (1) comprehensively evaluate CV function at rest and during maximal exercise and to validate the presence of CV dysfunction in a cohort of human subjects with MetS; (2) to evaluate the therapeutic value of aerobic exercise training for improving large elastic artery stiffening and arterial-ventricular coupling; and (3) to determine the effect of single-dose supplementation with RSV on AS and central systolic blood pressure in MetS and healthy controls. The central hypothesis of this collective work was that subclinical CV dysfunction develops as a result of co-occurring risk factors associated with MetS, and can be improved by the implementation of therapeutic lifestyle interventions including aerobic exercise training and nutritional supplementation with RSV. The rationale for the work presented within this document is based on the importance of reversing CV dysfunction associated with MetS for CVD risk reduction as well as to advance our understanding of the link between CVD and MetS.

MetS is defined by the co-occurrence of clinical risk factors and has become a principal health concern in the U.S. due to its association with CVD [1]. A systematic review of data from existing observational studies has determined that MetS is associated with a 2-fold increased risk in CV outcomes and a 1.5-fold increase in all-cause mortality [2]. Numerous studies have demonstrated LV dysfunction at rest in MetS [3-6], but not all [7, 8], suggesting that LV dysfunction may mediate, in part, the link between CV risk and MetS. Accordingly, the purpose of our initial efforts was to

complete a comprehensive assessment of LV and arterial structure and function at rest and during exercise in MetS vs. age- and sex-matched healthy controls. This study, presented in Chapter 2, was the first to provide a comparison of LV and arterial function responses at rest and during dynamic exercise in individuals with MetS but without T2DM and/or overt CVD and healthy controls. Exclusion of subjects with T2DM and symptomatic or clinical CVD was an important strength of this study and permitted the interpretation of our results to be a reflection of MetS rather than a consequence of conditions that may exacerbate LV dysfunction. Use of load-independent measures to assess LV contractility at rest eliminated a general shortcoming of many existing studies. Indeed, altered loading conditions, including abnormal resting HR and pathological LV chamber remodeling are key features of MetS and may influence load-dependent measures of systolic function. Under resting conditions we observed elevations in arterial parameters including cIMT and cfPWV (arterial stiffness; AS), and LVmass, which have been previously described in earlier investigations of MetS [8-10].

Literature suggests that changes in the mechanical and elastic properties of the large elastic arteries play a role in the underlying mechanism contributing to AS associated with MetS [11]. Stability and compliance of the large elastic arteries are dependent on the relative content of two important scaffolding proteins: collagen and elastin. An imbalance in the production and degradation of these two proteins contributes to large artery stiffness and may occur in response to inflammation and endothelial dysfunction [11].

Furthermore, load-independent assessments of LV contractility described in Chapter 2 revealed no evidence of contractile dysfunction in MetS. Exercise testing is a

well-established method to generate physiological stress in order to reveal pathological alterations not present at rest. During maximal exercise MetS demonstrated impaired LV systolic function, blunted systemic vasodilation, and reduced cardiac pump performance [12]. Collectively, LV contractile deficits contributed to a blunted arterial-ventricular coupling reserve and impaired peak aerobic capacity in MetS [12]. Taken together, these data demonstrate the capacity for the development of pathophysiological adaptations to CV structure and function in the presence of co-occurring CV risk factors prior to the development of chronic disease. Notably, these findings underscore the value of MetS as a prognostic tool for the identification of individuals at high risk for CVD but without evidence of clinical disease including T2DM and/or overt CVD. Importantly, these data formed the foundational work for investigations into the use of exercise training for the treatment of arterial stiffness in MetS without T2DM and/or overt CVD.

As detailed by assessment of arterial structure and function in Chapter 2, this work and others [12, 13], have demonstrated arterial dysfunction in MetS that is characterized by elevations in cfPWV, cIMT, carotid cross-sectional area, and carotid wall stress and tension. Notably, large elastic artery stiffening has been shown to modulate LV loading conditions through elevations in central systolic pressure [14]. Left ventricular hypertrophy (LVH) occurs as a maladaptive response to chronic elevations in central arterial pressure. Complications of LVH include diastolic heart failure, systolic heart failure, and impaired coronary flow reserve [4]. In Chapter 2 we describe evidence of early diastolic dysfunction during supine rest in MetS. Consequently, early recognition and identification of effective strategies for reducing AS may have important clinical

implications for individuals with MetS.

Aerobic exercise training has been shown by others to be an effective intervention to reduce AS in young and healthy and middle-aged and older individuals [15, 16]. Building on our previous findings detailed in Chapter 2, we investigated whether 8 weeks of aerobic exercise training is an effective strategy to reduce AS in MetS without T2DM and/or overt CVD. These investigations are detailed in Chapter 3 wherein we demonstrate a reduction in cfPWV in MetS after 8 weeks of aerobic exercise training. Importantly, improvements in AS occurred in the absence of significant reductions in weight, BMI, and metabolic profile (Table 3.3) suggesting that improvements in AS associated with aerobic exercise training are independent of other established benefits of exercise training. A lack of training effect on established outcomes of aerobic exercise training begs the question of whether or not a longer duration or combination diet and exercise is required to elicit improvements in these parameters; However, an investigation of the effects of altering the intensity or number of treatment strategies to target AS in MetS warrants consideration.

Central systolic blood pressure, rather than traditional brachial blood pressure, is known to provide a more accurate index of afterload; reflecting the pressure that the LV must generate in order to eject blood from the heart to the periphery. Using PWA for the non-invasive estimation of central pressure we demonstrated significant reductions in cSBP in MetS. This finding is of clinical importance, as it is known that cSBP is superior to brachial BP for the prediction of CV outcomes [17]. Furthermore, PWA revealed an increase in the sub-endocardial viability ratio (SEVR), an index of myocardial oxygen supply and demand. In the absence of a direct measurement of CV function by Doppler

echocardiography, changes in the SEVR indicate potential improvements in cardiac function after 8 weeks of aerobic exercise training in MetS. Whether or not these improvements in AS elicited by short-term exercise training in MetS will be maintained over a longer duration of time remains unknown. Recent evidence from existing literature suggests that in persons with MetS and T2DM, aerobic exercise training elicits initial improvements in AS, and these benefits are subsequently lost when training is continued for a longer duration [18]. Consequently, the capacity of aerobic exercise training to reduce AS may have a more lasting benefit for MetS without chronic disease; however, this proposal is merely speculative and cannot be supported by data presented within this document.

Understanding the mechanisms underlying the changes in arterial function associated with MetS is essential in the prevention or treatment of individuals with co-occurring risk factors for CVD. AS is determined by the composition of the extracellular matrix (elastin-collagen network) and vascular smooth muscle cell function [11, 19, 20]. Changes that occur in the expression and/or bioactivity of structural proteins in the arterial wall (and heart) lead to AS. Vascular remodeling of the large elastic arteries, including fibrosis and elastic fiber degradation results in decreased arterial compliance [21-23]. It is possible that changes in large artery structure may play a role in the pathophysiology of AS in MetS. Structural changes to the extracellular matrix of large arteries in MetS have been attributed to the activation of matrix metalloproteinases (MMPs), key regulators of arterial and cardiac remodeling [20, 24]. Activation of various MMPs in the carotid intima-media is associated with characteristics of large artery stiffening including increased fragmentation of elastin [25], overproduction of abnormal

collagen [20], increased vascular smooth muscle cell migration and proliferation in the carotid intima-media layer [26, 27]. In animal models, increased MMP9 activity, responsible for the degradation of elastin and collagen [25], was directly linked with AS [28] and cardiac stiffness [29]. MMP activation in MetS may be further exacerbated by decreased activity of endogenous tissue inhibitor of metalloproteinases (TIMPs). In hypertensive patients, plasma concentrations of MMP9 and TIMP1 were elevated vs. controls and are implicated in AS and associated with an increased risk of CV events [30-32]. Notably, TIMP1 inhibits MMP1 activity that increases type 1 collagen (high tensile strength and rigidity) formation. MMP/TIMP activity is altered in MetS, with higher concentrations of MMP2, 8, 9 and TIMP1 vs. controls [33-35]. The ratio of MMP/TIMP activity plays a central role in remodeling of the extracellular matrix and thus, may mediate structural changes of the large arteries in the presence of a chronic pro-inflammatory environment as observed in MetS. As detailed in Chapter 3 we measured plasma markers of tissue remodeling (MMPs and TIMPs) and found that exercise training was able to produce favorable reductions in circulating MMP1 and MMP7, both of which were found to have positive associations with cfPWV [36].

Expanding on structural changes that occur to the extracellular matrix in MetS that may contribute to AS, additional material properties of the vasculature can play a role in arterial stiffening, as well. Among these properties the most important contributors to large artery stiffness include increased synthesis of collagen, non-enzymatic glycation of collagen to form crosslinks, and vascular smooth muscle cell hypertrophy [20]. Under hyperglycemic conditions, proteins and lipids in the vasculature may undergo non-enzymatic glycation and oxidation steps that trigger molecular

transformations resulting in the formation of advanced glycation end products (AGEs). Elevated levels of oxidative stress and the hyperglycemic environment associated with MetS promotes the formation and accumulation of advanced glycation end products (AGEs) which may play a causative role in large artery stiffening [37]. Indeed, animal models of T2DM and MetS have demonstrated significant associations between arterial stiffness and enhanced AGE accumulation in conduit arteries [38]. AGE accumulation in the vasculature contributes to alterations in extracellular and intracellular structure and function through a variety of mechanisms, including crosslink formation between proteins in the basement membrane of the extracellular matrix, and through the modification of cellular function through the activation of AGEs with the cell surface receptor for AGEs [39]. Crosslink formation of proteins in the extracellular matrix can alter properties of large structural matrix proteins including type I collagen and elastin which fosters reduced arterial elasticity and vascular stiffening. In the endothelium, AGEs reduce the bioavailability and activity of endothelium-derived NO and prostacyclin and may enhance expression of the vasoconstrictor, endothelin-1 [40]. The fundamental determinants of AGE formation include the rate of turnover of proteins for glycoxidation, the degree of hyperglycemia, and the level of oxidant stress [41] and thus, AGE formation and accumulation is accelerated in MetS and T2DM and likely contributes to the pathophysiology of arterial stiffening observed in MetS. While we did not quantify AGE deposition in the studies presented within this dissertation, this measurement would provide an enhanced understanding of the underlying mechanisms contributing to AS associated with MetS. Taken together, the results detailed in Chapter 3 suggest that

aerobic exercise training holds promise as an effective treatment approach for targeting AS in MetS without chronic disease.

Next, we built upon our previous work by broadening our investigation of the therapeutic potential of aerobic exercise training for reducing CVD risk in MetS. Evidence from Chapter 2 demonstrated that individuals afflicted with MetS display not only deleterious adaptations to arterial structure and function at rest, but also demonstrate deficiencies during exercise. Specifically, MetS exhibited impaired coupling between the heart and the arterial system, an interaction termed arterial ventricular coupling (E_a/E_{es}). Additionally, a blunted decrease in E_a/E_{es} during exercise in MetS was shown to coincide with a significantly reduced exercise capacity. Collectively, these results suggest an impaired CV response to physiological stress produced during aerobic exercise in MetS. In Chapter 4 we determine the effects of 8 weeks of aerobic exercise training on E_a/E_{es} during peak exercise in MetS without T2DM or clinical indications of CVD. In this investigation we demonstrate that 8 weeks of aerobic exercise training was not only able to improve, but to restore peak arterial-ventricular coupling, peak LV contractility, and aerobic capacity in MetS to levels observed in healthy untrained controls. The key finding of this study was that improvements in peak exercise arterial-ventricular coupling in MetS, which was due directly to improvements in peak LV end-systolic elastance (E_{es}), a load-independent index of the contractility and systolic stiffness of the LV. Not surprisingly, 8 weeks of aerobic exercise training failed to improve resting LV structure, diastolic dysfunction, metabolic profile, or body composition. The lack of an observed effect is most likely due to the short exercise training duration. Indeed, others have shown that several months of aerobic training

alter deleterious LV remodeling [42, 43]. Improvements in arterial-ventricular coupling were accompanied also by a modest increase in exercise capacity as measured by $\text{VO}_{2\text{peak}}$. It was determined that improvements in Ea/Ees during exercise contributed, to a small degree, to improvements in exercise capacity, however, it is likely that this improvement can also be explained by other exercise-induced adaptations not considered by this study. Nevertheless, improvements in aerobic capacity likely reflect a reduced CVD risk in MetS. Our initial investigations in Chapter 2 determined that the blunted decrease of arterial-ventricular coupling in MetS is due not only to impaired LV contractility but also to a blunted peripheral vasodilation in response to exercise. In Chapter 4, we proposed that improvements in LV contractility were mediated, in part, by improvements in SV, afterload, and Ea/Ees. In support of this hypothesis, we demonstrated improvements in SV but observed no change in the Ea component of arterial-ventricular coupling, which was in contrast to observed reductions in peak vascular resistance after exercise training in MetS. Importantly, Ea represents an index of the net arterial load imposed on the LV that integrates individual elements of arterial load including, but not limited to, peripheral vascular resistance. For this reason, a lack of change in Ea does not rule out the potential for improvements in other principal components of arterial load. As a final point, we reported a lack of effect of exercise training on body composition or clinical blood biomarkers, which was in agreement with our findings described in Chapter 3. Taken together, this study demonstrated that 8 weeks of aerobic exercise training of moderate-to-high intensity was sufficient to improve peak exercise arterial-ventricular coupling, LV contractility, reduce peripheral vascular resistance, and increase aerobic capacity in MetS without T2DM and/or overt

CVD.

In Chapters 3 and 4, aerobic exercise training was reported to have beneficial effects on AS and the interaction between the arteries and the heart in individuals with MetS [36, 44]. Favorable effects of exercise training on modulating CV function in MetS may be a result of underlying improvements in inflammation and autonomic nervous system activity. MetS is characterized by elevated glucose levels and chronic, low-grade inflammation [45]. Hyperglycemia and excess cytokine secretion increase oxidative stress [46], which reduces NO bioavailability [47] and may exert an acute and chronic influence on AS [48]. Increased large artery stiffness is associated with normative aging and is an independent predictor for an increased risk for stroke, coronary artery disease, and heart failure [10]. In individuals with metabolic disease, such as MetS, the interaction of AS with other CV risk factors (i.e. hypertension, obesity, and dyslipidemia) accelerates the vascular aging process [49] that results in large artery stiffening. Recent literature has shed light on the importance of inflammation in the pathogenesis of AS.

The activation of MMPs and TIMPs, regulators of tissue remodeling, is mediated, in part, by the renin-angiotensin system, oxidative stress, endothelial dysfunction, and increased expression of pro-inflammatory cytokines and cellular adhesion molecules [50, 51]. MetS is associated with a pro-inflammatory state, which favors endothelial dysfunction and impaired vasodilation [21-23]. Impaired relaxation of the smooth muscle is associated with diminished production or availability of vasodilator substances, particularly NO, due to a decline in the production of NO by the endothelium, inactivation of NO by reactive oxygen species (ROS), or a decline in the availability of

co-factors required for the synthesis of NO. In the presence of cardiovascular risk factors (e.g. hypertension, T2DM, hyperglycemia, etc.) the normal production of NO by eNOS is altered such that eNOS favors the generation of ROS including superoxide, and hydrogen peroxide. Generation of ROS stimulates the continued production of ROS creating a positive feedback loop leading to chronic elevations in oxidative stress and a reduction in NO bioavailability [39]. Moreover, inflammation may directly diminish NO bioavailability through the production of ROS, which effectively augments oxidative stress and promotes the upregulation of inflammation [39].

In addition, the local production of hormones that play a role in the structural modification of the arteries has been implicated in the development of AS. Specifically, AS has been linked to increased activity of ANG II, which is responsible for vascular smooth muscle cell hypertrophy, increased production of collagen, activation of MMPs and increased production of ROS and other inflammatory cytokines [39]. The release of inflammatory cytokines stimulates the production of C-reactive protein (CRP) by vascular smooth muscle cells. CRP plays an indirect role in the pathology of arterial stiffness through its direct promotion of endothelial dysfunction [52]. Taken together, these findings suggest an association between the role of inflammation, oxidative stress, and endothelial dysfunction in the pathology of impaired arterial elasticity in MetS.

Structural changes that may play a role in arterial dysfunction associated with MetS may take several months to develop, however AS may also be altered acutely through changes in VSMC tone, which is regulated by sympathetic nervous system (SNS) activity and endothelial cell function [53]. The SNS is known to play a key role in

the short-term regulation of vasomotor tone and blood pressure in order to maintain cardiovascular homeostasis [54]. Recent evidence suggests that sympathetic activity and vascular function may be linked and more specifically demonstrate that the activity of the SNS may modulate functional properties of large arteries [54]. In terms of large artery stiffness, sympathetic activity may alter AS through an indirect change in arterial pressure or through an active increase in vascular tone, peripheral vasoconstriction and decreased arterial diameter [54]. Data from human-based research has demonstrated associations between increased sympathetic discharge and large artery stiffness[54]. It is notable that the association between sympathetic activity and large artery stiffness may be bidirectional. Large artery stiffness may impair carotid baroreflex sensitivity while conversely, increased sympathetic discharge may play a role in the modulation of arterial stiffness. Although we did not use a direct measure of SNS activity in the studies included in this document, exercise training has previously been reported to improve autonomic control in obese women [55] which may represent a potential mechanism by which aerobic exercise training can illicit improvements in AS in MetS.

Current guidelines for the treatment of MetS suggest therapeutic lifestyle changes including both diet and physical activity. Chapters 2-4 demonstrate the capacity for improvements in CV function in MetS following short-term exercise training. This would suggest that preservation of CV improvements over time requires maintenance of lifestyle changes specifically, regular physical activity. Other strategies for the treatment of MetS include pharmacological or dietary interventions that focus on improving NO bioavailability, oxidant stress, inhibit the renin-angiotensin system, and target AGE cross-links. Nutritional supplementation represents an attractive therapeutic

intervention for alleviating CV dysfunction in MetS as it is effortless to integrate and maintain. Chapter 5 demonstrates a divergence from the use of exercise-training to induce improvements in MetS and describes the initial efforts by our laboratory to investigate the therapeutic potential for RSV, a naturally occurring nutritional supplement, to induce acute changes in AS and BP in MetS. In addition to changes in the structural components of the arterial wall that manifest over a period of time, short-term changes may occur to mediate vascular stiffness. Specifically, changes in VSMC tone and SNS activity have been demonstrated to modulate AS in an acute fashion [56-59]. Evidence from human [60, 61] and animal [62-64] research demonstrate the potential of RSV for CV protection based on the ability of RSV to acutely reduce inflammation and oxidative stress, two mechanisms mediating large artery stiffness associated with MetS [56-59].

Building on evidence from studies examining the effects of dietary supplements on arterial compliance we hypothesized that a single dose of RSV would elicit acute improvements in AS and central blood pressure in MetS. For example, high dietary intake of isoflavones is associated with a reduced PWV in healthy individuals [20]. Further, supplementation with curcumin, the active ingredient in turmeric, a spice that is used frequently in Indian cuisine has been shown to improved AS in young rodent models [11]. Similarly, in aged animals, 4 weeks of supplementation with curcumin was associated with reduced superoxide bioavailability and whole artery AGE expression. Taken together, these findings provide support for the use of dietary supplementation as an effective intervention for improving AS in individuals in MetS.

Results from Chapter 5 led us to fail to reject the null hypothesis that an acute

dose of 500mg or 1000mg of RSV does not alter AS or central BP at 45 or 75 minutes post-supplementation in MetS and we are unable to accept the alternative hypothesis that single-dose RSV supplementation can improve AS and central BP at 45 and 75 minutes post-supplementation in MetS. In an effort to reconcile these findings we suggest several limitations that may have contributed to our results. These limitations are reviewed in detail in Chapter 5. While we were unable to provide evidence in support of the therapeutic potential of RSV to improve CVD risk through AS and BP, improvements in study design and recruitment are encouraged for building upon this preliminary data and are reviewed in Chapter 7.

In conclusion, our data provides a comprehensive examination of the consequences to CV function in MetS prior to evidence of T2DM and/or chronic CVD. Further, we perform initial evaluations of both physical and nutritional changes in lifestyle as strategies for improving CV dysfunction and subsequent risk for CVD in MetS. The findings of this dissertation are in agreement with the central hypothesis that subclinical CV dysfunction at rest and during exercise occurs in the presence of MetS without T2DM and/or over CVD, and that this dysfunction can be improved by implementation of therapeutic lifestyle interventions, including aerobic exercise training. While we cannot apply this conclusion to evidence presented within this document for the therapeutic potential of chronic nutritional supplementation with RSV, we believe future studies may provide the evidence-based support necessary to extend these conclusions to supplementation with RSV in MetS. The results of the studies contained within this dissertation underscore the importance of establishing targeted strategies for reversing CV function at rest and during exercise in MetS.

Reference List

1. Malik, S., et al., *Impact of the Metabolic Syndrome on Mortality From Coronary Heart Disease, Cardiovascular Disease, and All Causes in United States Adults*. Circulation, 2004. **110**(10): p. 1245-1250.
2. Mottillo, S., et al., *The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis*. J Am Coll Cardiol, 2010. **56**(14): p. 1113-32.
3. Aijaz, B., et al., *Abnormal Cardiac Structure and Function in the Metabolic Syndrome: A Population-Based Study*. Mayo Clinic Proceedings, 2008. **83**(12): p. 1350-1357.
4. Azevedo, A., et al., *Increasing number of components of the metabolic syndrome and cardiac structural and functional abnormalities - cross-sectional study of the general population*. BMC Cardiovascular Disorders, 2007. **7**(1): p. 17.
5. Mahmud, A., et al., *Left ventricular structural and functional changes in the metabolic syndrome*. J Cardiometab Syndr, 2009. **4**(2): p. 81-8.
6. Pagé, A., et al., *Metabolic Syndrome Is Associated With More Pronounced Impairment of Left Ventricle Geometry and Function in Patients With Calcific Aortic Stenosis: A Substudy of the ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin)*. Journal of the American College of Cardiology, 2010. **55**(17): p. 1867-1874.
7. Chinali, M., et al., *Comparison of cardiac structure and function in American Indians with and without the metabolic syndrome (the Strong Heart Study)*. Am J Cardiol, 2004. **93**(1): p. 40-4.

8. de las Fuentes, L., et al., *Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass*. Eur Heart J, 2007. **28**(5): p. 553-9.
9. Lin, H.-F., et al., *The risk of the metabolic syndrome on carotid thickness and stiffness: Sex and age specific effects*. Atherosclerosis, 2010. **210**(1): p. 155-159.
10. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. **43**(8): p. 1388-95.
11. Fleenor, B.S., *Large elastic artery stiffness with aging: novel translational mechanisms and interventions*. Aging Dis, 2013. **4**(2): p. 76-83.
12. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. Exp Physiol, 2013.
13. Scuteri, A., et al., *The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study*. Eur Heart J, 2010. **31**(5): p. 602-13.
14. Marti, C.N., et al., *Endothelial dysfunction, arterial stiffness, and heart failure*. J Am Coll Cardiol, 2012. **60**(16): p. 1455-69.
15. Tanaka, H., et al., *Aging, habitual exercise, and dynamic arterial compliance*. Circulation, 2000. **102**(11): p. 1270-5.
16. Currie, K.D., S.G. Thomas, and J.M. Goodman, *Effects of short-term endurance exercise training on vascular function in young males*. Eur J Appl Physiol, 2009. **107**(2): p. 211-8.
17. Wang, K.L., et al., *Central or peripheral systolic or pulse pressure: which best relates to target organs and future mortality?* J Hypertens, 2009. **27**(3): p. 461-7.

18. Madden, K.M., et al., *Aerobic training-induced improvements in arterial stiffness are not sustained in older adults with multiple cardiovascular risk factors*. J Hum Hypertens, 2013. **27**(5): p. 335-9.
19. Dao, H.H., et al., *Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension*. Cardiovasc Res, 2005. **66**(2): p. 307-17.
20. Ziemann, S.J., V. Melenovsky, and D.A. Kass, *Mechanisms, pathophysiology, and therapy of arterial stiffness*. Arterioscler Thromb Vasc Biol, 2005. **25**(5): p. 932-43.
21. Boos, C.J. and G.Y. Lip, *Elevated high-sensitive C-reactive protein, large arterial stiffness and atherosclerosis: a relationship between inflammation and hypertension?* J Hum Hypertens, 2005. **19**(7): p. 511-3.
22. Pasceri, V., et al., *Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs*. Circulation, 2001. **103**(21): p. 2531-4.
23. Pasceri, V., J.T. Willerson, and E.T. Yeh, *Direct proinflammatory effect of C-reactive protein on human endothelial cells*. Circulation, 2000. **102**(18): p. 2165-8.
24. Jacob, M.P., *Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions*. Biomed Pharmacother, 2003. **57**(5-6): p. 195-202.
25. Rodriguez-Pla, A., et al., *Metalloproteinase-2 and-9 in giant cell arteritis - Involvement in vascular remodeling*. Circulation, 2005. **112**(2): p. 264-269.

26. Pauly, R.R., et al., *Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation*. Circ Res, 1994. **75**(1): p. 41-54.
27. Wang, M. and E.G. Lakatta, *Altered regulation of matrix metalloproteinase-2 in aortic remodeling during aging*. Hypertension, 2002. **39**(4): p. 865-73.
28. Bouvet, C., et al., *Sequential activation of matrix metalloproteinase 9 and transforming growth factor beta in arterial elastocalcinosis*. Arterioscler Thromb Vasc Biol, 2008. **28**(5): p. 856-62.
29. Ammarguella, F.Z., et al., *Fibrosis, matrix metalloproteinases, and inflammation in the heart of DOCA-salt hypertensive rats: role of ET(A) receptors*. Hypertension, 2002. **39**(2 Pt 2): p. 679-84.
30. Laviades, C., et al., *Abnormalities of the extracellular degradation of collagen type I in essential hypertension*. Circulation, 1998. **98**(6): p. 535-40.
31. Tan, J., et al., *Impact of the metalloproteinase-9/tissue inhibitor of metalloproteinase-1 system on large arterial stiffness in patients with essential hypertension*. Hypertens Res, 2007. **30**(10): p. 959-63.
32. Tayebjee, M.H., et al., *Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)*. Am J Hypertens, 2004. **17**(9): p. 764-9.
33. Cicero, A.F., et al., *Vascular remodeling and prothrombotic markers in subjects affected by familial combined hyperlipidemia and/or metabolic syndrome in*

- primary prevention for cardiovascular disease*. Endothelium, 2007. **14**(4-5): p. 193-8.
34. Goncalves, F.M., et al., *Increased circulating levels of matrix metalloproteinase (MMP)-8, MMP-9, and pro-inflammatory markers in patients with metabolic syndrome*. Clin Chim Acta, 2009. **403**(1-2): p. 173-7.
 35. Miksztowicz, V., et al., *Increased plasma activity of metalloproteinase 2 in women with metabolic syndrome*. Metabolism, 2008. **57**(11): p. 1493-6.
 36. Donley, D.A., et al., *Aerobic Exercise Training Reduces Arterial Stiffness in Metabolic Syndrome*. J Appl Physiol (1985), 2014.
 37. Singh, R., et al., *Advanced glycation end-products: a review*. Diabetologia, 2001. **44**(2): p. 129-46.
 38. Reddy, G.K., *AGE-related cross-linking of collagen is associated with aortic wall matrix stiffness in the pathogenesis of drug-induced diabetes in rats*. Microvasc Res, 2004. **68**(2): p. 132-42.
 39. Chantler, P.D. and J.C. Frisbee, *Arterial function in cardio-metabolic diseases: from the microcirculation to the large conduits*. Prog Cardiovasc Dis, 2015. **57**(5): p. 489-96.
 40. Goldin, A., et al., *Advanced glycation end products: sparking the development of diabetic vascular injury*. Circulation, 2006. **114**(6): p. 597-605.
 41. Brownlee, M., A. Cerami, and H. Vlassara, *Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications*. N Engl J Med, 1988. **318**(20): p. 1315-21.

42. Fujimoto, N., et al., *Cardiovascular effects of 1 year of progressive and vigorous exercise training in previously sedentary individuals older than 65 years of age*. Circulation, 2010. **122**(18): p. 1797-805.
43. Turner, M.J., et al., *Effect of endurance exercise training on left ventricular size and remodeling in older adults with hypertension*. J Gerontol A Biol Sci Med Sci, 2000. **55**(4): p. M245-51.
44. Fournier, S.B., et al., *Improved Arterial-Ventricular Coupling in Metabolic Syndrome after Exercise Training*. Med Sci Sports Exerc, 2014.
45. Conen, D., et al., *Metabolic syndrome, inflammation, and risk of symptomatic peripheral artery disease in women: a prospective study*. Circulation, 2009. **120**(12): p. 1041-7.
46. Evans, J.L., et al., *Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes*. Endocr Rev, 2002. **23**(5): p. 599-622.
47. Zhang, H., et al., *Role of TNF-alpha in vascular dysfunction*. Clin Sci (Lond), 2009. **116**(3): p. 219-30.
48. Xu, C., et al., *Hypercholesterolemia superimposed by experimental hypertension induces differential distribution of collagen and elastin*. Arterioscler Thromb Vasc Biol, 2000. **20**(12): p. 2566-72.
49. Lee, H.Y. and B.H. Oh, *Aging and arterial stiffness*. Circ J, 2010. **74**(11): p. 2257-62.
50. Galis, Z.S. and J.J. Khatrri, *Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly*. Circ Res, 2002. **90**(3): p. 251-62.

51. Wang, M., et al., *Proinflammatory profile within the grossly normal aged human aortic wall*. Hypertension, 2007. **50**(1): p. 219-27.
52. Park, S. and E.G. Lakatta, *Role of inflammation in the pathogenesis of arterial stiffness*. Yonsei Med J, 2012. **53**(2): p. 258-61.
53. Wilkinson, I.B. and C.M. McEniery, *Arterial stiffness, endothelial function and novel pharmacological approaches*. Clin Exp Pharmacol Physiol, 2004. **31**(11): p. 795-9.
54. Palatini, P., et al., *Arterial stiffness, central hemodynamics, and cardiovascular risk in hypertension*. Vasc Health Risk Manag, 2011. **7**: p. 725-39.
55. Trombetta, I.C., et al., *Weight loss improves neurovascular and muscle metaboreflex control in obesity*. Am J Physiol Heart Circ Physiol, 2003. **285**(3): p. H974-82.
56. Leikert, J.F., et al., *Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells*. Circulation, 2002. **106**(13): p. 1614-7.
57. Olas, B. and B. Wachowicz, *Resveratrol and vitamin C as antioxidants in blood platelets*. Thromb Res, 2002. **106**(2): p. 143-8.
58. Sonnett, T.E., et al., *Diabetes mellitus, inflammation, obesity: proposed treatment pathways for current and future therapies*. Ann Pharmacother, 2010. **44**(4): p. 701-11.
59. Wallerath, T., et al., *Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase*. Circulation, 2002. **106**(13): p. 1652-8.

60. Carrizzo, A., et al., *Resveratrol improves vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism*. Hypertension, 2013. **62**(2): p. 359-66.
61. Wong, R.H., et al., *Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure*. Nutr Metab Cardiovasc Dis, 2011. **21**(11): p. 851-6.
62. Fiori, J.L., et al., *Resveratrol prevents beta-cell dedifferentiation in nonhuman primates given a high-fat/high-sugar diet*. Diabetes, 2013. **62**(10): p. 3500-13.
63. Mattison, J.A., et al., *Resveratrol prevents high fat/sucrose diet-induced central arterial wall inflammation and stiffening in nonhuman primates*. Cell Metab, 2014. **20**(1): p. 183-90.
64. Rivera, L., et al., *Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats*. Biochem Pharmacol, 2009. **77**(6): p. 1053-63.

Chapter 7

Future Directions

Future Directions

Metabolic syndrome is a multifaceted disease state characterized by multiple co-occurring risk factors. Clinical manifestation of MetS carries a profound risk for CVD that surpasses the sum of the individual CVD risk carried by each component [1]. MetS can lead to deleterious alterations to CV structure and function even in the absence of T2DM and overt CVD [2-4]. Current recommendations from the NIH for the treatment of MetS include adoption of healthy lifestyle changes including, but not limited to, regular physical activity and a heart healthy diet [5]. Preventing or slowing the progression and onset of T2DM is one of the primary goals of treating MetS, second only to CVD risk reduction [5], however, the ideal treatment or treatment combination for long-term improvements in CVD risk and prevention of T2DM in MetS without chronic CVD is largely unknown. Future studies in animals and humans examining the long-term effects of lifestyle changes in MetS would provide a greater understanding of the underlying pathological mechanisms of MetS.

Investigations outlined in Chapter 3-4 of this dissertation provide initial evidence for the promise of short-term aerobic exercise training for improving AS, LV contractility, and coupling of the heart and arteries in MetS without T2DM and/or CVD. Future studies should focus on addressing the important limitations reported in these chapters as well as build upon the foundation that this work provides. In particular, future studies to address the effects of exercise training on AS in MetS should focus on uncovering the mechanisms responsible for reported improvements. Specifically, a non-invasive assessment of autonomic function using Finapres technology could provide a way to

determine the role of sympathetic activity in reductions in cfPWV that accompany short-term aerobic exercise. Due to the lack of an observed effect on metabolic plasma markers, future studies should consider a longer exercise training duration or higher intensity training to identify whether longer duration or higher intensity can exert additional benefits to individuals afflicted with MetS. Studies of longer duration would have the added advantage of determining whether the benefits associated with short-term aerobic exercise can be maintained over time. Most importantly, future longitudinal studies, which have the benefit of being able to establish a sequence of events, should be conducted to examine the ability of long term exercise training to prevent or delay the progression to T2DM and CVD.

Furthermore, due to a lack of observed effect of exercise training on arterial load (as indicated by no pre to post change in Ea in Chapter 3) in MetS, the use of other assessments of arterial function including characteristic impedance and cIMT may provide evidence for a more conclusive effect of exercise training on arterial structure/function.

One common challenge in human-based subject research is achieving large and equal numbers of both male and female participants, as is evidenced by data presented within this document. Unequal representation in gender within current literature should be a key consideration for the planning of future studies. Potential investigations into the consequence of sex differences on the effects of exercise training and nutritional supplementation may have an important impact on the interpretation of conclusions from existing literature, and for the development of target treatment strategies for MetS.

A further complication of research surrounding MetS is the inherent complexity of co-occurring risk factors. For example, by considering MetS to be a single co-morbid condition as the studies presented in this document do, one may overlook the importance of different combinations of the individual components of MetS, and how these combinations respond to treatment strategies. Future studies using MetS should attempt to examine how different clusters of MetS components impact CV function and progression to T2DM and overt CVD in MetS. Additionally, the use of a continuous scale to classify MetS may allow insight to how metabolic disease severity affects CV function and also response to treatment.

For a more mechanistic understanding of the CV dysfunction underlying MetS, future research may be best conducted in animal models of MetS, such as the obese Zucker fatty. Animal models of MetS may allow for a more detailed study of the contribution of changes in inflammatory status and oxidant stress to treatment for MetS. Furthermore, animal models of disease represent a more controlled and cost-effective starting point for determining efficacy of various treatment options for MetS, especially future investigations of nutritional supplementation.

The overall objective of this dissertation was to define CV dysfunction in the metabolic syndrome and to identify practical therapeutic strategies to improve CV dysfunction, specifically to target large artery stiffness and coupling between the arteries and heart; therefore, future directions should focus on achieving a more thorough understanding of the CV risk associated with MetS and whether long-term therapeutic lifestyle change (physical and nutritional) can prevent the progression of MetS to T2DM and clinical CVD.

Reference List

1. Rosin, B.L., *The progression of cardiovascular risk to cardiovascular disease*. Rev Cardiovasc Med, 2007. 8 Suppl 4: p. S3-8.
2. Fournier, S.B., et al., *Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome*. Exp Physiol, 2013.
3. Scuteri, A., et al., *Arterial stiffness and influences of the metabolic syndrome: a cross-countries study*. Atherosclerosis, 2014. 233(2): p. 654-60.
4. Scuteri, A., et al., *Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness*. J Am Coll Cardiol, 2004. 43(8): p. 1388-95.
5. Grundy, S.M., et al., *Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement*. Circulation, 2005. 112(17): p. 2735-52.
6. Lalande, S., R.J. Petrella, and J.K. Shoemaker, *Effect of exercise training on diastolic function in metabolic syndrome*. Applied Physiology, Nutrition, and Metabolism, 2012. 38(5): p. 545-550.
7. Fujimoto, N., et al., *Cardiovascular Effects of 1 Year of Progressive and Vigorous Exercise Training in Previously Sedentary Individuals Older Than 65 Years of Age*. Circulation, 2010. 122(18): p. 1797-1805.
8. Lakatta, E.G. and D. Levy, *Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease*. Circulation, 2003. 107(2): p. 346-54.

9. Hartley, L.H., et al., *Physical Training in Sedentary Middle-aged and Older Men III. Cardiac Output and Gas Exchange at Submaximal and Maximal Exercise*. Scandinavian Journal of Clinical & Laboratory Investigation, 1969. 24(4): p. 335-344.
10. Seals, D.R., et al., *Endurance training in older men and women. I. Cardiovascular responses to exercise*. Journal of Applied Physiology, 1984. 57(4): p. 1024-1029.
11. Beere, P.A., et al., *Aerobic Exercise Training Can Reverse Age-Related Peripheral Circulatory Changes in Healthy Older Men*. Circulation, 1999. 100(10): p. 1085-1094.
12. Schulman, S.P., et al., *Continuum of Cardiovascular Performance Across a Broad Range of Fitness Levels in Healthy Older Men*. Circulation, 1996. 94(3): p. 359-367.
13. Aronson, D., *Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes*. J Hypertens, 2003. 21(1): p. 3-12.

Curriculum Vitae

Sara B. Fournier

West Virginia University School of Medicine
Department of Exercise Physiology
P.O. Box 9227
Morgantown, WV 26506

H: 802-309-4933

W: 304-293-8878

Email: sfournier@hsc.wvu.edu

EDUCATION

PhD, Exercise Physiology

2009-2015

Department of Exercise Physiology
West Virginia University, Morgantown, WV

B.S., magna cum laude, Chemistry

2005-2009

Saint Michael's College, Colchester, VT

RESEARCH EXPERIENCE

West Virginia University

2011-2015

Graduate Research Assistant in Exercise Physiology, 2009-Present

Doctoral Research: Initiated the conception, design, and execution of human-based research focused on the assessment of life-style and dietary interventional strategies on the structure and function of the cardiovascular system.

- Prepared, presented, and communicated research at multidisciplinary national conferences: Experimental Biology, 2011-2013 and The ACSM Annual Meeting, World Congress on Exercise is Medicine, 2014.
- Planned and actively participated in all recruitment initiatives for human-subject research.
- Managed the selection of participants and the scheduling of all pre and post study visits for each research participant.
- Organized and actively participated in data collection, recording, data interpretation and analysis for all human-subject research.
- Nurtured participant involvement to foster subject adherence and fulfillment.

TEACHING EXPERIENCE

Guest Lecturer – BMS 706

2014

West Virginia University, Morgantown, WV

Presented a lecture to Biomedical Science doctoral students titled, *“Human Cardiovascular Exercise Physiology Research”* highlighting human-based cardiovascular research at WVU.

Guest Lecturer – Exph 680

2013

West Virginia University, Morgantown, WV

Presented a lecture to Exercise Physiology Masters students titled, *“Arterial stiffness and pulse wave analysis – their role in enhancing cardiovascular assessment.”*

Laboratory Teaching Assistant – General Chemistry

2007

Saint Michael's College, Colchester, VT

Organized laboratory preparation, met with students upon request and graded written work.

PROFESSIONAL EXPERIENCE

Intern – Quality Control Laboratory

2006-2009

Mylan Technologies Inc., St. Albans, VT

Gained a sound understanding of applicable cGMP regulations and expectations, and developed an understanding and expertise in HPLC, transdermal drug design, and routine physical and chemical testing.

FELLOWSHIPS AND AWARDS

Outstanding Doctoral Student, Exercise Physiology, WVU

2015

Sponsored Fellow, Training Grant-5T32HL090610-04, WVU

2011- 2014

WVU Biomedical Sciences Graduate Student Travel Award

2013

First Place Oral Presentation. Clinical Science. 2013 E.J. Van Liere Memorial Convocation & HSC Research Day, WVU

2013

First Place Poster Presentation. Basic Science 2009 I. 2012 E.J. Van Liere Memorial Convocation & HSC Research Day, WVU

2012

Outstanding Senior Student Chemistry Department Award, SMC

2009

HONOR SOCIETIES

Alpha Nu Chapter of Delta Epsilon Sigma, National Scholastic Honor Society

2008

PRESENTATIONS AT NATIONAL MEETINGS

Lee K, DeVallance E, **Fournier SB**, Brainard, C, White E, Miller WC, Dino G, and Chantler, PD. The Effects of a Community-Based Multi-lifestyle Intervention on CV Health in Rural Populations. Experimental Biology national meeting 2015

E DeVallance, **S Fournier**, K LeMaster, K Lee, D Bonner, D Donley, PD Chantler. Safety Of Resistance Training with Elevated Arterial Stiffness. Experimental Biology national meeting 2015

K LeMaster, E DeVallance, **S Fournier**, K Lee, D Bonner, D Donley, and PD Chantler. Predicting Hypertensive Responses to Exercise in Women. Experimental Biology national meeting 2015.

K LeMaster, E DeVallance, **S Fournier**, K Lee, D Bonner, D Donley, and PD Chantler. The Effects of Resistance Training on Carotid Stiffness in Patients with the Metabolic Syndrome: A Pilot Study. Experimental Biology national meeting 2015.

C, Moore, **SB Fournier**, E DeVallance, K Lee, D Bonner, D Donley, L Sherlock, PD. Chantler. Deep Water Exercise Training is Associated with Decreased Arterial Stiffening in Women. Experimental Biology national meeting 2015.

Fournier SB, DeVallance ER, Donley DA, Bonner DE, Chantler PD., (2015) Resveratrol Augments Aerobic Exercise Training-Induced Improvements in VO₂: a pilot study. Van Liere Research Day. West Virginia University, 2015.

Petrone AB, Barr TL, Devlin K, **Fournier SB**, DeVallance ED, Lee K, Chantler, PD. Matrix Metalloproteinase Levels May Mediate Ischemic Stroke Incidence and Recovery in Stroke Patients with Metabolic Syndrome. AHA International Stroke Conference, Nashville Tennessee, February 2015.

Sara B. Fournier, David A. Donley, Jefferson C. Frisbee, Stephen E. Always, FACSM, Paul D. Chantler. Acute Effect of Resveratrol on Measures of Arterial Function in Metabolic Syndrome. ACSM National Conference, 2014.

E. DeVallance, **SB Fournier**, JT Butcher, JC Frisbee, PD Chantler. Altered Wall Mechanics of Conduit Arteries of Obese Zucker Rats With Progression of Metabolic Syndrome. Experimental Biology, San Diego, CA 2014.

Chantler, PD., **Fournier, SB.**, Fancher, IS., DeVallance, ER., Rottgen, TS., Nida, CA., Bell, S., and Dick, GM. Acute effects of dietary bisphenol A (BPA) on endothelial function in humans. Submitted to Experimental Biology, San Diego, CA 2014.

CS Moore, L Sherlock, K Lee, DA Donley, **SB Fournier**, ER DeVallance, PD Chantler. Shallow Water Aerobics is Associated with Decreased Arterial Stiffening. Van Liere Research Day, West Virginia University, 2014.

EM Casto, **SB Fournier**, ER DeVallance, GM Dick, PD Chantler. The Effect of Bisphenol A on Human Endothelial Function. Van Liere Research Day, West Virginia University, 2014.

K Branyan, **SB Fournier**, ER DeVallance, K Lee, DA Donley, PD Chantler. The Impact of Insulin Resistance on Arterial Stiffness and Aerobic Capacity. Van Liere Research Day, West Virginia University, 2014.

NK Mickinac, **SB Fournier**, K Lee, ER DeVallance, DA Donley, SE Always, PD Chantler. Increasing Endothelial Function by Utilizing the Acute Effects of Resveratrol Consumption. Van Liere Research Day, West Virginia University, 2014.

K Lee, **SB Fournier**, ER DeVallance, DA Donley, DE Bonner, PD Chantler. Effect of Aerobic Exercise Training on Matrix Metalloproteinase in Patients with Metabolic Syndrome. Van Liere Research Day, West Virginia University, 2014.

Paul D Chantler, Ibra S Fancher, **Sara B Fournier**, Evan DeVallance, Trey S Rottgen, Corey A Nida, Suzanne Bell, Gregory M Dick. Acute Effects of Dietary bisphenol A on Arterial Health. American Heart Association Scientific Sessions, Dallas, TX 2013.

Pearman, M., DeVallance, E., **Fournier, S.**, Bonner, D., Donley, D., Warden, B., Gahrib, W., Chantler, P. Does Arterial Health Influence Exercise Tolerance? ACSM National Conference, 2013.

Sara B Fournier, David A Donley¹Brian L Reger, Daniel E Bonner, Bradford E Warden, Wissam Gharib, Paul D Chantler. Is resting diastolic dysfunction related to impaired cardiovascular reserve in the metabolic syndrome? Experimental Biology, Boston, MA 2013.

Fournier SB, Donley DA, Reger B, Bonner DE, Warden BE, Gahrib, W, Chantler, PD., (2013) Impaired Contractile Reserve Is Associated With Depressed Diastolic Function In Metabolic Disease. Van Liere Research Day, West Virginia University, 2013.

DeVallance ER, Pearman ME, **Fournier SB**, Donley D, Bonner D and Chantler PD., (2013) Abnormal Exercising Central Systolic Pressure Response in High Risk Metabolic Disease Individuals. Van Liere Research Day, West Virginia University, 2013.

Pearman, M., DeVallance, E., **Fournier, S.**, Bonner, D., Donley, D., Warden, B., Gharib, W., Chantler, PD., Examining the relationship between arterial function and VO₂peak. Van Liere Research Day, West Virginia University, 2013.

Pearman, M.; DeVallance, E.; **Fournier, S.**; Bonner, D.; Warden, B. E.; Gharib, W.; Donley, D.; and Chantler, P. (2013) "Relationships between Peak Oxygen Uptake and Arterial Function: a Preliminary Study," *International Journal of Exercise Science: Conference Abstract Submissions*: Vol. 9: Iss. 1, Article 62.

Sara B. Fournier; Brian Reger; David A. Donley, MSc; Daniel E. Bonner, MSc; Brad Warden, MD; Deepak Hooda, MD; I Mark Olfert, PhD; Paul D. Chantler, PhD. Increased Metabolic Risk is Linked to Abnormalities in Arterial-Ventricular Interactions. Van Liere Research Day, West Virginia University, 2012.

Sara B Fournier, Brian Reger, David A Donley, MSc, Daniel E Bonner, MSc, Bradford E Warden, MD, Paul D Chantler, PhD. Improving Arterial-Ventricular Coupling Reserve in Patients with Metabolic Disorders through Exercise Training. Experimental Biology, San Diego, CA 2012.

O'Leary, HA, Kothur, A, **Fournier, SB**, Goodwill, AG, Frisbee, JC & Brock, RW. Effect of remote ischemic preconditioning on hepatic parenchymal and microvascular damage in obesity. FASEB J March 17, 2011 25:1117.9

PRESENTATIONS AT INTERNATIONAL MEETINGS

DeVallance E, Lemaster K, **Fournier SB**, Lee K, Bonner D, Donley D, and Chantler PD. Does Resistance Training Elevated Arterial Stiffness in Patients with the Metabolic Syndrome? International Obesity Summit, London England April 2015

PUBLICATIONS

Fournier, SB., Reger, BL., Donley, DA., Bonner, DE., Warden, BE., Gharib, W., Frisbee, JC., Olfert, IM., Chantler, PD. Improved Arterial-Ventricular Coupling Reserve in Metabolic Syndrome After Aerobic Exercise Training. *Medicine & Science in Sports & Exercise*, 2014.

Donley DA*, **Fournier SB***, Reger B, DeVallance, E., Bonner DE, Olfert, IM, Frisbee, JC, Chantler PD., Aerobic Exercise Training Reduces Arterial Stiffness in Metabolic Syndrome. J Appl Physiol (1985), 2014.

*These authors contributed equally to this work.

Sherlock, LA, **SB Fournier**, E. DeVallance, S. Carte, PD Chantler. Effects of Shallow Water Aerobic Exercise Training on Arterial Stiffness and Pulse Wave Analysis in Older Individuals: November 2014; 8 (4).

De Vallance ER., **Fournier SB.**, Donley DA., Bonner DE., Lee K., Frisbee JC., Chantler PD., Exploring the Cardiovascular Dysfunction with Obesity: Is it due to Adiposity or Cardiovascular Risk Factors? International Journal of Obesity, 2014.

Fournier SB, Reger BL, Donley DA, Bonner DE, Warden BE, Gharib W, Failing CF, Olfert MD, Frisbee JC, Olfert IM, Chantler PD., Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome. Experimental Physiology 2013. 99 (1), 149-163

UNIVERSITY SERVICE

Assisted in graduate student recruitment **2010-2012**
West Virginia University, Morgantown, WV
Coordinated and participated in events for graduate student recruitment.

Trunk-or-Treat **2013**
West Virginia University, Morgantown, WV
Promoting science education as a representative of the Biomedical Graduate Student Organization handing out candy to local children.

PROFESSIONAL MEMBERSHIPS

American College of Sports Medicine (Student Member)
American Heart Association (Student Member)
American Physiological Society (Student Member)
Microcirculation (Student Member)

SKILLS AND TECHNIQUES

Software: Proficient in MS Word, PowerPoint, Excel, SPSS, EndNote, and experience using Photoshop.

Benchtop Techniques: Vascular ultrasound, Luminex, blood collection/processing, HPLC, SphygmoCor Technology, and maximal exercise testing.