SKELETAL MUSCLE BLOOD FLOW RESPONSES TO EXERCISE IN METABOLIC SYNDROME

By

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I. GENERAL INTRODUCTION

Metabolic syndrome (MS) is present in nearly one third of the United States population. MS increases a person’s risk of developing cardiovascular disease and diabetes. Physical activity is an effective non-pharmacological way to combat the syndrome. Research in animal models of MS suggests blood flow responses to exercise are impaired due to altered vascular control mechanisms. Impaired blood flow and/or altered vascular control during exercise can adversely affect oxygen delivery, metabolic waste removal, glucose disposal, and blood pressure regulation – all of which may be especially detrimental in this population and may accelerate development of cardiovascular disease and type 2 diabetes. In addition, impairments may lead to greater muscle fatigue, reduced exercise tolerance, and aversion to physical activity.

Research in animal models of MS support the presence of impaired functional vasodilation and altered vascular control mechanisms, resulting in reduced exercise capacity. Whether such findings translate to human MS has yet to be assessed. The overall goal of this dissertation was to gain insight into exercise blood flow control in human MS. The specific aims were to determine: 1) whether adult humans with MS exhibit blunted exercise-induced skeletal muscle blood flow, 2) whether blood flow responses in MS are attenuated with additional physiological stress, 3) whether adults with MS exhibit increased α-adrenergic vasoconstriction during exercise, and 4) whether α-adrenergic vasoconstriction during exercise is related to muscle sympathetic nerve activity.
We hypothesized skeletal muscle blood flow during exercise would be lower in adults with MS when compared with healthy controls. Additionally, we hypothesized the rise in exercise blood flow due to hypoxia would be blunted in adults with MS. The exact mechanisms behind such differences are unknown. Based on experimental evidence, we hypothesized adults with MS would exhibit increased \( \alpha \)-adrenergic vasoconstriction during exercise. Lastly, we hypothesized this level of vasoconstriction during exercise would be positively related to muscle sympathetic nerve activity.

To test these hypotheses, we measured forearm blood flow (Doppler ultrasound) during dynamic forearm exercise in adults with MS and healthy controls. The first project measured vascular responses under both normoxic and hypoxic conditions. The second project directly and specifically assessed adrenergic responsiveness using intra-arterial infusion of \( \alpha \)-adrenergic receptor agonists. Muscle sympathetic nerve activity (microneurography of the peroneal nerve) was measured to explore the link between sympathetic nerve activity, vascular responsiveness, and blood flow control during exercise. The projects included in this dissertation further our understanding of the pathophysiology of the syndrome and highlight the importance of integrative vascular control in both health and disease.
I. BACKGROUND

Clinical Perspective

Adults with metabolic syndrome (MS) are obese, dislipidemic, hypertensive, hyperglycemic and are at high risk for developing cardiovascular disease and diabetes (4, 26, 78-81). Physical activity is a common non-pharmacological way to combat the syndrome (26, 80, 138, 251). If the normal rise in muscle blood flow with exercise is limited in MS, this can adversely affect oxygen delivery, metabolic waste removal, and glucose disposal—all of which may be especially detrimental in this population. To provide better treatments toward reversing cardiovascular impairments and improving individual health, it is important to elucidate whether differences in exercise muscle blood flow exist in MS humans and the mechanisms behind these potential impairments.

Skeletal Muscle Blood Flow

Blood flow delivered to skeletal muscle navigates through an extensive vascular tree. After leaving the heart, large conduit vessels branch into feed arteries external to the muscle tissue. Upon entrance into skeletal muscle, feed arteries divide into arteriolar networks; these networks are directly exposed to vasoactive stimuli from skeletal muscle fibers and determine the magnitude and distribution of blood flow within the muscle (35, 204, 221).
Anatomical features of skeletal muscle arterioles are important to blood flow control. The innermost layer of the arteriole consists of endothelial cells positioned longitudinally, which sense and respond to changes in shear forces (37, 203). The external wall provides circumferential force by aligning smooth muscle cells perpendicular to the axis of flow (37, 38). Complex interactions between skeletal muscle fibers, smooth muscle cells, endothelial cells, and perivascular nerves regulate skeletal muscle blood flow (203, 205).

Blood flow is primarily regulated by changes in vessel resistance. Vascular conductance, the inverse of resistance, is directly proportional to the radius of the vessel raised to the fourth power (51, 226). Vessel radius can be modified by various signaling mechanisms. A unique balance of vasodilatory and vasoconstrictor influences act directly on smooth muscle or indirectly through the vascular endothelium to determine skeletal muscle blood flow (226).

**Control of Skeletal Muscle Blood Flow Responses to Exercise**

A close relationship exists between skeletal muscle metabolic rate and oxygen delivery (9, 51, 104, 187). At rest, skeletal muscle perfusion is low and with exercise onset, blood flow increases in proportion to muscle oxygen demand (9, 104, 192, 224). This increase in flow (exercise hyperemia) is mediated by five local control systems: humoral, endothelial, mechanical, metabolic, neural (51, 147). Each category is described in more detail below, with primary focus on results from forearm exercise research in humans.
It is important to recognize multiple categories may be active at any given time; additionally many share overlapping vascular control substances. For example, nitric oxide (NO, a potent vasodilator) plays separate roles in flow-mediated, neural, and humoral control of exercise hyperemia (39, 68, 157, 224). In addition, any attempt to quantify the role of a single factor will be altered by synergistic interactions. Thus, it is difficult to quantify contributions of each specific factor.

**Humoral Control of Blood Flow**

Substances in the blood are known to dictate changes in skeletal muscle perfusion. As oxygen is removed from the red blood cell, S-nitrosohemoglobin (NO bound to hemoglobin) is released; in addition, deoxygenated hemoglobin can reduce plasma nitrate to NO—both evoking smooth muscle relaxation (37, 39, 68, 126). Red blood cells have also been shown to release adenosine triphosphate (ATP) in response to hemoglobin deoxygenation (11, 70). ATP acts on endothelial purinergic (P2Y) receptors to induce NO and prostacyclin production and release, causing smooth muscle relaxation and resultant vasodilation (21, 37, 68, 69, 209). At rest, ATP does not appear to play a primary role in maintaining blood flow (64); however, experimental evidence supports its importance in both immediate and steady-state exercise vasodilation (64, 105, 132, 191, 243). Other humoral factors (i.e. angiotensin II, vasopressin, atrial natriuretic peptide, etc) may also play a role in steady-state exercise blood flow, although research is currently limited in humans.
**Mechanical Control of Blood Flow**

Mechanical effects of muscle contraction elicit rapid increases in skeletal muscle blood flow (39, 40, 44, 225, 227). The magnitude of vasodilation is proportional to the strength of contraction and does not appear to be mediated by neural or local metabolic mechanisms (15, 24, 44, 165). At least a portion of this hyperemia can be attributed to mechanically-induced potassium channel opening and smooth muscle hyperpolarization (8, 37, 103). Mechanical influences on vascular control are greatest during low-to-moderate intensity single muscle contractions; the contribution becomes less important during repeated contractions seen during dynamic exercise (131).

**Endothelial Control of Blood Flow**

Endothelial cells respond to chemical substances within the blood as well as forces imparted on blood vessel walls (i.e. shear, vessel stretch); in response to these signals, endothelial-derived factors (i.e. NO, prostacyclin, endothelial-derived hyperpolarizing factor/EDHF) are released to alter vascular tone (51, 198). For example, an increase in shear will elevate intracellular (endothelial) free calcium, signaling the release of vasodilating prostaglandins and NO from endothelial cells (47, 136). These dilator systems interact in a redundant fashion. Research supports a significant contribution of NO (~20%) to steady-state exercise blood flow (201). However, NO is not obligatory and exercise hyperemia can occur in its absence. Prostaglandins are known to contribute only modestly (~12%) and transiently to forearm exercise hyperemia (201). Taken together, other vasodilatory factors must be present to restore and/or maintain blood flow responses to exercise.
Metabolic Control of Blood Flow

Metabolic rate plays an important role in determining skeletal muscle blood flow during steady-state exercise (9, 104, 148, 192, 224). Metabolites from actively contracting muscle diffuse toward resistance arterioles, causing vasodilation to increase oxygen delivery (51, 148). A host of metabolites are capable of modulating changes in blood flow during exercise and it is unlikely a single metabolite produces all vasodilation observed. More likely, several factors contribute to variable extents depending on muscle fiber type, exercise intensity, and time after initiation of exercise (51, 148). Potential metabolites include, but are not limited to: oxygen, lactate, adenosine, ATP, potassium (39, 148).

Adenosine is formed extracellularly during skeletal muscle contraction and levels in the interstitium increase even at low exercise intensities (113, 198). However, adenosine does not appear to be obligatory for exercise hyperemia at low-to-moderate workloads and makes limited contributions during heavier exercise in the human forearm (158). Interestingly, there is a “bimodal distribution” of adenosine vasodilator responsiveness; that is, only a fraction of participants studied exhibit adenosine-mediated vasodilation (158). This finding supports the potential for heterogeneity in vascular control.

Neural Control of Blood Flow

Neural control of blood flow works primarily through the release of norepinephrine from sympathetic nerve terminals. Norepinephrine-mediated activation of post-junctional α-adrenergic receptors causes vascular smooth muscle contraction (13, 221). Whereas
norepinephrine is the primary neurotransmitter in humans, non-adrenergic neurotransmitters (ATP and NPY) may also contribute to sympathetic vasoconstriction by respectively binding purinergic (P2x) and neuropeptide Y receptors (NPY Y1R) on vascular smooth muscle (19, 106).

Sympathetic tone plays an important role in determining exercise muscle blood flow by restricting conducted vasodilation (109, 224). Despite increases in sympathetic nerve activity with exercise, a given amount of sympathetic activation evokes less vasoconstriction in the active muscles as exercise intensity increases (termed “functional sympatholysis”) (148, 184, 192). The result is diminished sympathetically-mediated vasoconstriction in active muscle, yet preserved constriction in inactive tissues. This regional heterogeneity is graded with exercise intensity and optimizes blood flow to metabolically active muscles through increases in muscle metabolites (i.e. adenosine, potassium, lactate) and other sympatholytic factors (i.e. NO, prostacyclin) (190, 220, 228).

Factors such as NO and vasodilating prostaglandins do not appear to blunt sympathetic vasoconstriction on their own and combined inhibition augments vasoconstriction by only ~10% (58); thus other factors must be necessary to redistribute flow to metabolically active muscles. Whereas increases in sympathetic nerve activity promote the release of epinephrine from the adrenal medulla, β2-adrenergic vasodilation is not known to play an important role in functional sympatholysis (221). Interestingly, evidence suggests ATP may be capable of abolishing post-junctional α-adrenergic
mediated vasoconstriction in the forearm of young adults (132). ATP is also 
sympatholytic in middle-aged adults, but to a lesser extent (219). Thus middle-aged 
persons must rely on factors other than ATP to oppose α-adrenergic vasoconstriction, 
supporting the concept of both redundant and heterogeneous vascular control 
mechanisms.

**Summary:** A variety of local vascular control mechanisms, none of which appear to be 
obligatory, work together to match oxygen delivery with metabolic demand. Multiple 
 factors may be active at any given time (i.e. neural and metabolic) and many share 
overlapping vascular control substances (i.e. ATP, potassium, NO). Inhibition or 
impairment in one mechanism may be compensated for by another to maintain steady-
state blood flow during exercise.

**Hypoxia-mediated Vasodilation**
Systemic hypoxia leads to a reduction in arterial oxygen content (137, 189). This 
reduction in the arterial partial pressure of oxygen elicits carotid chemoreceptor-
mediated increases in sympathetic nerve activity (196, 213). To preserve oxygen 
consumption under hypoxic conditions, an increase in oxygen extraction and/or 
compensatory peripheral vasodilation occurs (22, 28, 137, 189); the result is a balance 
of local vasodilation with sympathetically-mediated vasoconstriction to ultimately 
During hypoxic exercise, α-adrenoceptor responsiveness to a given amount of norepinephrine is maintained (241). Given an increase in sympathetic nerve activity with hypoxia, enhanced vasodilator signals must be present to increase blood flow and maintain oxygen delivery (59, 241). Activation of β₂-adrenoceptors contributes to approximately half (~50%) of hypoxic vasodilation at rest and during mild intensity exercise (237, 240). However, the relative importance of the β-adrenergic component decreases with increased exercise intensity (240). Thus, other signals must contribute at higher intensities to ensure appropriate oxygen delivery during hypoxia.

Experimental evidence suggests NO contributes to compensatory vasodilation with hypoxia, although sources differ with intensity. Specifically, during lower intensity exercise, NO contributes to compensatory dilation via β₂-adrenergic activation; however, with increasing intensity, NO-contribution is independent of β-adrenoceptors (28). Research suggests NO may work together with vasodilating prostaglandins to explain ~50% of compensatory vasodilation at higher intensities (46). Additionally, as described earlier, red blood cells promote ATP-mediated NO release from the vascular endothelium in response to reduced oxygen content; however the importance of ATP to compensatory dilation during systemic hypoxia has yet to be assessed. Surprisingly, whereas adenosine has been thought to play an important role in hypoxic vasodilation (151), it does not appear to be a primary factor in the rise of forearm skeletal muscle blood flow with hypoxia either at rest or during exercise in humans (28, 29).
Summary: Hypoxia provides an enhanced metabolic signal relative to exercise alone that challenges oxygen delivery and alters sympathetic tone. Experimental evidence suggests vascular responses to hypoxia share many pathways with dynamic exercise. The coordination of these control mechanisms varies with exercise intensity to ultimately determine the degree of compensatory vasodilation.

Metabolic Syndrome

In 1998 the World Health Organization coined the term “Metabolic Syndrome (MS)” and today nearly one third of the United States population meets diagnostic criteria (81). MS is a primary health concern affecting both sexes and all ethnic groups (26, 78-80). Adults are characterized as having MS if they meet at least three of the following National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria as modified by the American Diabetes Association: central obesity (waist circumference $>88$ cm females, $>102$ cm males), pre-hypertension (resting blood pressure $\geq 130/\geq 85$ mmHg), hypertriglyceridemia (triglycerides $\geq 150$ mg.dL$^{-1}$), hyperglycemia (fasting glucose $\geq 100$ mg.dL$^{-1}$ ) and/or dyslipidemia (HDL $<50$ mg.dL$^{-1}$ females, $<40$ mg.dL$^{-1}$ males) (4, 98). Adults with MS are at increased risk of developing cardiovascular disease, coronary heart disease, myocardial infarction, and type 2 diabetes (4, 26, 78-80).

Reduced physical activity is an important determinant of MS (80). Individuals are encouraged to participate in regular activity to prevent the development of MS and
progression toward diabetes and cardiovascular disease (26, 80, 138, 251). However, very little is known about physiological responses to exercise in this population.

**Summary**: Nearly one third of the United States population has MS and is at increased risk of developing cardiovascular disease and diabetes. Individuals are encouraged to participate in physical activity to combat the syndrome. However, very little is known about physiological processes that occur in adults with MS during physical activity.

**Skeletal Muscle Blood Flow in Animal Models of Metabolic Syndrome**

The majority of experimental evidence concerning vascular responses to physiological stressors in MS has come from animals. Animal models of MS, such as the zucker diabetic fatty (ZDF) and obese zucker rats (OZR), have greatly contributed to the understanding of the pathophysiology of the disease. Both animal models exhibit inactive leptin receptors resulting in hyperphagia, obesity, hypertension, hyperglycemia and dyslipidemia (45, 94). Results from these models consistently support blunted functional vasodilation in response to simulated exercise (87, 90, 92, 115, 166, 202, 248-250). Physiological mechanisms behind this functional impairment in animal models have been studied extensively.

**Structural Adaptations**

Animal models of MS exhibit structural remodeling of the microvasculature. Specifically, these animals present with narrowing of individual vessels, thinner vessel walls, smaller lumens, and reduced distensibility—all of which may contribute to increased vascular
resistance (88, 212). Other research supports increased vascular smooth muscle cell proliferation with MS (188). Additionally, microvascular rarefaction (reduced microvessel density) has been shown to lead to impaired whole-limb blood flow altered oxygen diffusion capacity in these animals (85, 88, 146).

**Humoral Control of Blood Flow**

A large portion of both human and animal models of MS exhibit insulin resistance. Experimental evidence from the ZDF rat suggests insulin is capable of inhibiting oxygen-dependent ATP release from the red blood cell, blunting ATP-mediated vasodilation (67). Thus, high levels of circulating insulin as a result of insulin resistance potentially contribute to impaired oxygen delivery in ZDF rats (67). Given ATP may be sympatholytic (132), blunted ATP-mediated vasodilation may result in enhanced sympathetic vasoconstriction during exercise in MS; however, this has yet to be examined.

**Mechanical Control of Blood Flow**

Resistance arteries from skeletal muscles in the OZR exhibit increased myogenic activation (93). This suggests mechanical factors associated with contraction may negatively impact vasodilation in MS. Mechanistically, rapid onset vasodilation is known to be dependent upon potassium channel opening (8), and OZR exhibit impaired potassium channel mediated vasodilation (115). Thus, it is reasonable to propose contraction-induced rapid vasodilation will be blunted in MS. However, it is unlikely this impairment will alter steady-state exercise blood flow due to redundancy in vascular
control mechanisms and the reduced importance of mechanical factors during steady-state exercise (131).

**Endothelial Control of Blood Flow**

Endothelial dysfunction in MS results in attenuated release and blunted responsiveness to endothelium-derived relaxing factors (i.e. prostaglandin, NO, EDHF). The OZR exhibits reduced prostacyclin synthesis (114), impaired hyperemic responses to prostacyclin analogs (87, 94, 250) and elevated levels of vasoconstrictor thromboxane (91, 95, 97). In addition, K\textsubscript{ATP} channels involved in both prostacyclin- and exercise-induced vasodilation are less active in OZR (115).

NO-mediated dilation has also been shown to be significantly reduced in OZR compared with control animals (94, 121, 242). This impaired dilation may be due to reduced sensitivity of vessels to NO or a reduction in the bioavailability of NO. Reduced NO bioavailability may be due to enhanced oxidative stress and free radical scavenging of NO commonly seen in MS (6, 93). In addition, the availability of tetrahydrobiopterin (BH\textsubscript{4}, a necessary cofactor for NO production) and L-arginine (a substrate of NO) are reduced in animal models of MS (31, 99, 160). Taken together, endothelial dysfunction likely contributes to impaired functional vasodilation in animal models of MS.

**Neural Control of Blood Flow**

Muscle sympathetic nerve activity (as determined pharmacologically) has been shown to be greater in OZR when compared with healthy control animals (25). The ultimate
effect of sympathetic nerve activity on the vasculature depends on both the strength of the activity and the vascular responsiveness to that activity. Chronic sympathetic activation—commonly observed in MS—can result in altered neurotransmitter release, receptor number, receptor sensitivity, and/or downstream signaling (50, 100, 101, 154, 163). Along these lines, research has shown obese women to exhibit reduced norepinephrine spillover in forearm skeletal muscle when compared with lean controls (43). In addition, animal models of diabetes and hypertension support the presence of hyperactive pre-synaptic α₂-adrenergic receptors and/or reduced norepinephrine overflow (20, 96, 217).

Results from both intact and isolated vessel preparations suggest animal models of MS exhibit enhanced basal adrenergic tone and/or increased α-adrenergic mediated vasoconstriction at rest (25, 84, 90, 150, 166, 211). During simulated exercise at mild and moderate intensities, an increase in α-adrenergic vasoconstriction in OZR contributes to reduced blood flow in the gastrocnemius muscle (84, 87, 90). Given functional sympatholysis is graded with intensity, α-adrenergic vasoconstriction was not seen to impair functional vasodilation at higher intensity contractions (84, 87, 90). Taken together, increased α-adrenergic mediated vasoconstriction contributes to impaired functional vasodilation during low-to-moderate intensity exercise. However, with increasing intensity and more severe metabolic strain, the impact of elevated adrenergic activity on functional vasodilation is diminished (i.e. functional sympatholysis) (84, 166) and other factors (i.e. non-adrenergic constriction, endothelial dysfunction, etc) may play a more direct role in reduced exercise blood flow responses.
Summary: Animal models of MS exhibit blunted functional vasodilation in response to simulated exercise. This impairment is likely due to a combination of increased sympathetic-mediated vasoconstriction and blunted vasodilatory mechanisms (i.e. ATP, prostacyclin, NO).

**Skeletal Muscle Blood Flow in Human Metabolic Syndrome**

Despite novel findings in animal models, the impact of MS on exercise blood flow has not been systematically addressed in humans. Thus, it remains unknown whether adults with MS exhibit muscle blood flow limitations during dynamic exercise. However, research from other hypersympathetic populations (i.e. aging, diabetes, etc) provides strong indication for impaired exercise hyperemia in human MS.

**Whole Limb Blood Flow in Human Obesity**

When blood flow responses to exercise are assessed in otherwise healthy obese humans, exercise hyperemia appears to be maintained. Research from our lab suggests blood flow responses to both dynamic forearm and leg exercise are preserved with obesity (152). Similar conclusions have been made during isometric leg exercise (102, 208). Thus, without the presence of other cardiovascular disease risk factors, blood flow responses to exercise in human obesity appear preserved.

In contrast to otherwise healthy obesity, with earlier disease prevalence (i.e. childhood obesity) and/or advanced disease status (i.e. cardiovascular disease, type 2 diabetes), impairments in exercise blood flow are observed. Obese children and adolescents are
known to exhibit impaired skeletal muscle vasodilatory responses to handgrip exercise (186) and dynamic knee-extensor exercise (128). Leg blood flow responses to dynamic cycling exercise also appear impaired in adults with type 2 diabetes (130). In this diabetic cohort, researchers observed significant relationships between leg blood flow responses to exercise and fasted blood glucose levels, suggesting hyperglycemia may contribute to impaired exercise blood flow (130). Taken together, blood flow responses to exercise appear to be maintained in otherwise healthy obesity whereas impairments are observed with early disease onset and in conditions of increased cardiovascular disease risk.

**Endothelial Control of Blood Flow**

Endothelial dysfunction is considered an early event in atherogenesis and precedes the development of detectable cardiovascular disease (48, 142). Given such wide acceptance of endothelial dysfunction with obesity (1, 128, 161, 215, 223), it may be surprising steady-state blood flow responses to exercise are maintained (discussed above). However, it is important to recognize noninvasive measures of endothelial function (i.e. Flow-Mediated Dilation, FMD) are collected in large conduit vessels at rest (1, 128, 161, 215, 223) and may not be representative of alterations in specific physiological control mechanisms and/or vascular responses to exercise (176, 179). It is also possible: 1) results are confounded by age (given the majority of subjects studied were middle-aged), 2) endothelial impairments are disguised by redundant vascular control mechanisms, and/or 3) endothelial-mediated control plays a smaller role in exercise hyperemia when compared with other factors (i.e. neural control). Few studies
have directly assessed endothelial control in human MS, but those that have support the presence of dysfunction at rest (156, 210). Whether this dysfunction translates to impaired blood flow responses during steady-state exercise has yet to be assessed.

**Neural Control of Blood Flow**

Adult humans with MS exhibit increased basal muscle sympathetic nerve activity (MSNA) which is positively correlated with the number of cardiovascular disease risk factors expressed (73, 117, 141, 195). The mechanism behind this increase is unknown; however a rise in MSNA has been attributed to changes in central autonomic regulation, a stiffening of receptive fields within the carotid bodies, and changes in body composition and/or insulin sensitivity (72, 124, 143, 195, 199, 206, 232, 233). In regard to smooth muscle responses, chronic sympathetic activation has been shown to result in altered neurotransmitter release, receptor number, receptor sensitivity, and/or downstream signaling (50, 100, 101, 154, 163).

Recent findings at rest suggest vasoconstrictor responses to norepinephrine are inversely related to MSNA in young adults (33); those adults with high MSNA exhibit reduced α-adrenergic mediated vasoconstriction. Along these lines, older adults are less responsive to α-adrenergic agonists and exhibit blunted sympathetically-mediated vasoconstriction at rest when compared with younger adults –possibly the result of chronic increases in MSNA and adrenergic receptor downregulation (55, 56, 60). Paradoxically, older adults exhibit increased α-adrenergic vasoconstriction during exercise (i.e. blunted functional sympatholysis) (56, 60); similar results have been
observed in essential hypertension (234). Blunted functional sympatholysis may limit any increase in blood flow necessary to meet metabolic demand of the active tissue. The exact mechanisms behind this paradox are unknown; however, it is reasonable to speculate impairments are observed in response to an imbalance between sympathetically-mediated vasoconstriction and metabolic vasodilation.

**Summary:** With the progression toward cardiovascular disease, impairments in steady-state exercise blood flow are observed. This may be due to altered vascular control mechanisms (i.e. endothelial dysfunction, enhanced MSNA, altered adrenergic responsiveness, increased sympathetically-mediated vasoconstriction). However, whether blood flow is impaired in humans with MS and the mechanisms behind this potential impairment are currently unknown.

**Hypoxia-mediated Vasodilation in Metabolic Syndrome**

In response to reduced oxygen concentrations, isolated *gracilis* arteries from OZR exhibit impaired hypoxia-mediated vasodilation (86, 97). This may be the result of blunted $\beta_2$-mediated vasodilation (150), given the importance of $\beta_2$-adrenoceptors to hypoxic vasodilation in young healthy adults (237, 240). Hypoxia-mediated vasodilation has also been shown to be dependent upon combined NO and prostaglandin release from the vascular endothelium (86). However, hypoxic vasodilation in OZR is predominantly prostaglandin-mediated, with a reduced role for NO (86). With increased reliance on prostanoids, altered hypoxic-mediated vasodilation may be due to impaired vasodilatory mechanisms and/or increased release of vasoconstrictor metabolites (i.e.
thromboxane) (182, 218). Thus, the OZR relies on alternative vascular control mechanisms that may lead to impaired hypoxia-mediated compensatory vasodilation.

In older adult humans, hypoxia-mediated vasodilation is preserved during low intensity forearm exercise, although NO signaling may be impaired (30). Whereas alternative vascular control mechanisms may be capable of maintaining oxygen delivery during low intensity exercise, this impairment in NO-mediated vasodilation negatively impacts hypoxia-mediated vasodilation at a higher workload and blunted compensatory vasodilation is observed (30). It is reasonable to speculate older adults, similar to the OZR, rely on alternative vascular control mechanisms to maintain compensatory vasodilation at low workloads; however, with increased physiological stress these compensatory mechanisms fail to meet metabolic demand and blood flow impairments are observed.

**Summary**: To match oxygen delivery with metabolic demand under hypoxic conditions, compensatory vasodilation is observed. However, specific mechanisms important to compensatory vasodilation in healthy controls appear to be altered in human aging and animal models of MS. Combined with increased sympathetically-mediated vasoconstriction, this may negatively impact vasodilatory responses to hypoxia and lead to impaired blood flow. Whether this remains true in human MS has yet to be examined.
III. SPECIFIC AIMS AND HYPOTHESES

The rationale for the studies included in this dissertation is based on the following key observations:

- **Animal models of MS exhibit:**
  - Reduced blood flow in response to simulated exercise (i.e. functional vasodilation).
  - Reduced hypoxia-mediated vasodilation and increased reliance on alternative vascular control mechanisms.
  - Reduced endothelial-dependent vasodilation.
  - Increased sympathetic nerve activity, adrenergic responsiveness, and sympathetically-mediated restraint during simulated exercise.

- **Humans with MS exhibit:**
  - Endothelial dysfunction and increased MSNA.

- **Other hyper-sympathetic disorders in humans:**
  - Aging humans exhibit endothelial dysfunction and increased MSNA, resulting in reduced blood flow and/or altered vascular control during dynamic forearm exercise.
  - Aging humans exhibit reduced hypoxia-mediated vasodilation and altered vascular control mechanisms (i.e. reduced reliance on NO-mediated hypoxic vasodilation)
  - Aging humans and adults with essential hypertension exhibit increased sympathetically-mediated vasoconstriction during exercise.
• MSNA plays an important role in adrenergic receptor expression, sensitivity, downstream signaling, and resultant vasoconstriction.

The overall goal of this dissertation was to gain insight into exercise blood flow control in human metabolic syndrome (MS). The specific aims were as follows:

Aim 1: Determine whether adult humans with MS exhibit blunted exercise-induced skeletal muscle blood flow.

Hypothesis: We hypothesized exercise muscle blood flow would be lower in adults with MS when compared with healthy controls.

Aim 2: Determine whether blood flow responses in MS are attenuated with additional physiological stress.

Hypothesis: We hypothesized the rise in exercise blood flow due to hypoxia would be blunted in adults with MS when compared with healthy controls.

Aim 3: Determine whether adults with MS exhibit increased α-adrenergic responsiveness during exercise.

Hypothesis: We hypothesized adults with MS would exhibit increased adrenergic vasoconstriction to intra-arterial infusion of α-adrenergic agonists during exercise.

Aim 4: Determine whether α-adrenergic responsiveness during exercise is related to muscle sympathetic nerve activity.

Hypothesis: We hypothesized the degree of α-adrenergic vasoconstriction during exercise would be positively related to muscle sympathetic nerve activity.
To test these hypotheses, we measured forearm blood flow (Doppler ultrasound) during dynamic forearm exercise in adults with MS and age-matched healthy controls. The first project measured vascular responses under both normoxic and hypoxic conditions (Aims 1 and 2). The second project accessed adrenergic responsiveness during exercise using intra-arterial infusion of adrenergic receptor agonists. Additionally, muscle sympathetic nerve activity (microneurography of the peroneal nerve) was assessed to explore the link between increased sympathetic nerve activity, vascular responsiveness, and blood flow control (Aims 3 and 4).
BLOOD FLOW RESPONSES TO EXERCISE AND HYPOXIA IN HUMAN METABOLIC SYNDROME

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A selection of the data presented was published previously:


ABSTRACT

Background: This study was designed to test whether adults with metabolic syndrome (MS, n=8, 29±2 years) exhibit altered hyperemic responses to forearm exercise under normoxic and hypoxic conditions when compared with healthy controls (n=13, 29±3 years). We hypothesized exercise muscle blood flow would be lower and the rise in exercise blood flow due to hypoxia would be blunted in adults with MS when compared with healthy controls.

Methods: We measured forearm blood flow (FBF, Doppler Ultrasound) and arterial oxygen saturation (pulse oximetry) during rest and steady-state dynamic forearm exercise (20 contractions/min at 8 and 12 kg) under two conditions: normoxia (0.21 FiO₂, ~98% SpO₂) and hypoxia (~0.10 FiO₂, 80% SpO₂). Forearm vascular conductance (FVC) was calculated as FBF ÷ mean arterial blood pressure.

Results: Exercise increased FVC from resting levels in both groups (Main effect of exercise, p<0.01) with FVC levels greater in adults with MS when compared with healthy controls (Main effect of group, p<0.01). Hypoxia caused an additional increase in FVC that was not different between groups (Main effect of hypoxia, p=0.01; Interaction of group and hypoxia, p=0.30); responses to hypoxia were heterogeneous within and between groups. Reporting FVC responses to hypoxia as absolute (Δ) or relative (%Δ) changes led to similar conclusions.

Conclusion: These results demonstrate greater exercise-mediated vasodilation in MS adults when compared with controls. Group differences in vascular responses to hypoxia during exercise were not detected. Individual compensatory responses to hypoxia were variable, suggesting diversity in vascular control.
INTRODUCTION

Nearly one third of the United States population has metabolic syndrome (MS) and is at increased risk of developing cardiovascular disease and diabetes (4, 26, 78-81). Individuals are encouraged to participate in physical activity to combat the syndrome (26, 80, 138, 251); however, very little is known about physiological processes that occur in adults with MS during physical activity.

Animal models of MS exhibit blunted functional vasodilation in response to simulated exercise (90, 92, 115, 166, 202, 248-250). This impairment is likely due to a combination of increased α-adrenergic mediated vasoconstriction (86, 87, 94, 114, 250) and blunted vasodilatory mechanisms (i.e. ATP, prostacyclin, NO) (25, 84, 90, 150, 166, 211). Despite novel findings in animal models, the impact of MS on exercise blood flow has not been systematically addressed in humans. Thus it remains unknown whether adults with MS exhibit muscle blood flow limitations during dynamic exercise.

Hypoxia provides an enhanced metabolic signal relative to exercise alone that challenges oxygen delivery and alters sympathetic tone (213). To match oxygen delivery with metabolic demand under hypoxic conditions, compensatory vasodilation is observed (22, 28, 137, 189). Specific mechanisms important for compensatory vasodilation in healthy subjects (i.e. NO, prostacyclin) appear to be altered in animal models of MS (86, 97); when combined with increased sympathetically-mediated vasoconstriction (25, 84, 90, 150, 166, 211), this may negatively impact vasodilatory responses to hypoxia and lead to impaired blood flow. Consistent with this concept,
animal models of MS demonstrate impaired hypoxia-mediated vasodilation (86, 97); whether these findings translate to human MS has yet to be examined.

This study aimed to determine whether adult humans with MS exhibit blunted exercise-induced skeletal muscle blood flow and whether blood flow responses in MS are attenuated with additional physiological stress. We hypothesized exercise muscle blood flow would be lower and the rise in exercise blood flow due to hypoxia would be blunted in adults with MS when compared with healthy controls. To test these hypotheses, we measured forearm blood flow (Doppler ultrasound) during dynamic forearm exercise in adults with MS and age-matched healthy controls, assessing vascular responses under both normoxic and hypoxic conditions.
MATERIALS AND METHODS

Subjects
Two groups (MS, healthy controls) of relatively young (18-55 years) adult men and women from the University of Wisconsin – Madison campus and surrounding areas were recruited for participation in the current study using Human Subjects IRB-approved advertisements. All subjects completed a screening process in which physical activity and personal health history (including history of medications, and family history of cardiovascular disease) were assessed. All healthy control subjects were lean (waist circumference <102 cm for males and <88 cm for females), normotensive (resting blood pressure <130/<85 mmHg), and free from overt cardiovascular disease and diagnosed sleep apnea as judged from self-reported medical history (See Appendix D) and laboratory-measured blood lipid and glucose levels. All subjects were non-smokers and were not taking any cardiovascular medications, as determined by self-report. With regard to venous catheter risks, subjects who reported a general history of prolonged or difficult wound healing or any problems with bleeding or clotting were excluded in the screening process.

Adults were characterized as having MS if, after in-person assessment, subjects met at least three of the following National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria as modified by the American Diabetes Association: central obesity (waist circumference >102 cm males, >88 cm females), pre-hypertension (resting blood pressure ≥130/≥85 mmHg), hypertriglyceridemia
(triglycerides ≥150 mg/dL), hyperglycemia (fasting glucose ≥100 mg/dL) and/or dyslipidemia (HDL <40 mg/dL males, <50 mg/dL females) (4, 98).

Female subjects were premenopausal and all subjects had a negative urine pregnancy test on the study day. To avoid confounding effects of changes in female hormone levels (33, 153, 162) all women were studied during the early follicular phase/placebo phase (days 1-5) of the menstrual cycle, as determined by self-report (n=3 using hormonal birth control).

Subjects were instructed to refrain from exercise, non-steroidal anti-inflammatory drugs, alcohol, and caffeine for 24 -hours prior to the study visit. Written informed consent was obtained from all subjects prior to study procedures. All procedures were approved by the Institutional Review Board at the University of Wisconsin – Madison and conformed to the standards set by the Declaration of Helsinki. All testing was performed in the Bruno Balke Biodynamics Laboratory at the University of Wisconsin – Madison.

**Descriptive Measurements**

Body composition and central adiposity were determined by body mass index and waist circumference. Forearm volume was determined using water displacement (241) and lean forearm mass was estimated used linear regression analysis from whole body dual-energy x-ray absorptometry scans (DEXA; GE Lunar Prodigy; Milwaukee, WI) taken previously from 22 lean adults and 50 obese adults (Appendix C1). Forearm
Maximal Voluntary Contraction (MVC) of the non-dominant arm was determined as the average of the two highest measurements from 5 trials using a hand dynamometer.

After a minimum 8-hour fast, whole blood was collected and levels of HDL cholesterol, triglycerides, and glucose were measured immediately (CardioChek; PTS Panels; Indianapolis, IN, USA). Additional blood samples were collected in a subset of participants (Control n=5, MS n=5) and plasma was frozen at -80 degrees C to be analyzed for insulin levels (radioimmunoassay; Wisconsin National Primate Research Center; Madison, WI).

**Blood Flow Measurements**

Forearm blood flow (FBF) was measured using Doppler ultrasound (Vivid 7, General Electric; Milwaukee, WI). Blood flow was determined as the product of mean blood velocity (cm/s) and vessel cross sectional area ($\pi \times \text{radius}^2$ with radius measured in cm) and was then multiplied by 60 to convert from mL/s to ml/min. By measuring blood flow entering the forearm at the level of the brachial artery (proximal to exercising muscles), we observed changes occurring in downstream arterioles in response to exercise and environmental stimuli [following the assumption that vasodilation in the forearm resistance arteries would result in a necessary increase in blood flow through the upstream brachial artery (204)].

A 12 MHz linear array probe was placed approximately midway between the antecubital and axillary regions, medial to the *biceps brachii* muscle, with a probe insonation angle
of <60 degrees and the sample volume adjusted to cover the width of the artery using identical methods as described previously (200, 201, 241). The ultrasound probe operator continuously adjusted the probe position to maintain a fixed insonation angle, compensating for subjects’ movements during exercise. Pulse-wave velocity was continuously assessed (beat-to-beat) and results are reported in 30-second intervals to reduce contraction-induced variability in blood flow.

A commercial interface unit (Multigon Industries; Younkers, NY) processed the angle-corrected, intensity-weighted Doppler audio information from the GE Vivid ultrasound system into a flow velocity signal sampled in real time with signal-processing software (PowerLab, ADinstruments; Colorado Springs, CO). All hemodynamic data were digitized, stored on a computer at 400 Hz, and analyzed off-line using PowerLab. Post-processing using PowerLab’s Chart application package yielded mean blood velocities.

Artery diameters were obtained from B-mode video images and measurements typically resulted in loss of pulse wave signal for 15 seconds. To determine vessel cross sectional area, artery diameter was calculated as the median of five measurements in late diastole, between muscle contractions. Arterial diameter was measured in the part of the artery running perpendicular to the ultrasound beam, identified by strong wall signals in a longitudinal section of the artery (measuring the distance between near and far intima-media interfaces). All measurements were made off-line by a trained operator. These procedures are identical to those used in many laboratories (30, 46, 234).
Forearm Exercise

Each subject laid supine with the non-dominant arm extended to the side (approximately 90 degrees) at heart level. Dynamic and rhythmic, non-dominant forearm exercise required subjects squeeze and release two handles together 4-5 cm to raise and lower a weight over a pulley. Exercise was completed at a rate of 20 times per minute (at a duty cycle of 1 second contraction : 2 second relaxation) to the rhythm of a metronome. Mild to moderate workloads (8 and 12 kg, approximately 20 and 30% of maximum) were used to minimize systemic effects of exercise (i.e. increases in MSNA, baroreflex activation, etc) (231). Intensities were selected based on previous research showing similar metabolic need (and thus blood flow requirement) between groups at the same absolute exercise intensities (62, 246) and results from older adults showing hypoxic-mediated vasodilation to be impaired at moderate (~8 kg) workloads (28). This forearm exercise model is identical to that used in several laboratories (30, 46, 234).

Estimated Forearm Oxygen Delivery, Extraction and Consumption

A 25 mm, 20-gauge venous catheter (Smiths Medical International LTD, Lancashire, UK) was inserted in an antegrade fashion into the antecubital vein in the non-dominant, exercising arm by an experienced physician. The catheter was flushed continuously with saline at 1 mL/min. The catheter was used to take blood samples throughout the study. Duplicate venous blood samples (2-4 mL) were drawn in heparinized syringes at rest and at 3.5 minutes of dynamic forearm exercise following a 5 mL waste sample (which was disposed of). Results from blood samples are presented from a subset of
subjects (Control n=11, MS n=7) due to difficulty obtaining samples at all time points. All samples were placed on ice and analyzed within 2 hours of collection for measurements of pH and the partial pressures of oxygen (P\textsubscript{v}O\textsubscript{2}) and carbon dioxide (P\textsubscript{v}CO\textsubscript{2}) using a blood-gas analyzer calibrated with tonometered blood (ABL500; Radiometer, Copenhagen, Denmark). Pilot work showed no detectable difference between measurements analyzed 0.5 or 2.5 hours after collection (p>0.05, Appendix C2). All results were temperature-corrected using custom made software.

Oxygen consumption was calculated using the following equation: Blood flow (mL/min) x [arterial oxygen content (mL/mL) – venous oxygen content (mL/mL)]. Oxygen content was determined using the following equation: [Hemoglobin (g/dL) x 1.36 (mL O\textsubscript{2}/g Hb) x S\textsubscript{x}O\textsubscript{2} (%)] + [0.0031 x P\textsubscript{x}O\textsubscript{2} (Torr)] where P\textsubscript{x}O\textsubscript{2} represents the partial pressure of oxygen of arterial or venous blood (denoted as x), S\textsubscript{x}O\textsubscript{2} represents the percentage of oxygen saturation of the hemoglobin, and 1.36 and 0.0031 represent constants describing the amount of bound and dissolved oxygen in blood (mL O\textsubscript{2}), respectively. Whole blood was collected and hemoglobin levels were assessed, however results were unreliable; therefore, standard measures of hemoglobin were used across groups (14 g/dL). Arterial oxygen saturation (S\textsubscript{a}O\textsubscript{2}) and venous partial pressure of oxygen (P\textsubscript{v}O\textsubscript{2}) were measured and venous oxygen saturation (S\textsubscript{v}O\textsubscript{2}) and arterial partial pressure of oxygen (P\textsubscript{a}O\textsubscript{2}) were estimated using the oxyhemoglobin dissociation curve (129). To our knowledge, no evidence exists to suggest adults with MS exhibit an altered oxyhemoglobin dissociation curve.
Hypoxic Hypoxia

Subjects completed exercise under two conditions: normoxia (0.21 FiO₂, ~98% SpO₂) and hypoxia (~0.10 FiO₂, 80% SpO₂). Subjects were instrumented with a nose clip and mouthpiece. Subjects breathed through a low-resistance two-way non-rebreathing valve (model 2400, Hans Rudolph) for both normoxic and hypoxic trials. This system allowed for maintenance of constant dead space ventilation independent of study condition.

During normoxic trials, subjects breathed room air. During hypoxic trials, the level of inspired oxygen was titrated to achieve arterial oxygen saturation (SpO₂) of 80% as assessed by pulse oximetry on the dominant hand (Datex-Ohmeda; Helsinki, Finland) and verified on the forehead (Nellcor, N-595; Pleaston, CA, USA). Two separate gas tanks [(9% O₂ + 91% N₂); (5% CO₂ + 21% O₂ + 74% N₂)] were mixed using a blender (air-oxygen mixer, Puritan-Bennett; Los Angeles, CA) in attempt to maintain individual normoxic CO₂ levels (isocapnic) while achieving desired SpO₂ (80%).

Data were transferred to and analyzed on a metabolic cart (Medgraphics, Ultima PFX; St. Paul, MN). Inspiratory and expiratory flow rates, as well as inspired and expired gases were sampled at the mouth (MedGraphics, Ultima PFX; St. Paul, MN). All signals were displayed on a chart recorder (MedGraphics Breeze Suite; St. Paul, MN) and sampled at 75 Hz. Data were analyzed as an average of data from the last 30 seconds of rest and steady-state exercise for measures of minute ventilation and end-
tidal carbon dioxide levels in a subset of subjects (Control n=9, MS n=6; due to a data storage error).

**Protocol**

The study required one 60-minute screening visit and one 3-hour study visit. Visits were often completed in the same day and were otherwise separated by less than two weeks. During the screening visit and after providing informed consent, subjects completed a medical history questionnaire followed by anthropometric measuring and blood sampling to determine eligibility. On the study day, subjects lay supine for the insertion of the venous catheter. Over the next 2.5 hours, subjects experienced four (4) individual trials in random order. Blood flow was measured (Doppler ultrasound) during normoxic and hypoxic conditions at rest and during dynamic forearm exercise (8 and 12 kg). Blood pressure was measured by an electronic sphygmomanometer (Datex-Ohmeda; Helsinki, Finland) on the upper arm and changes in blood pressure were confirmed using finger photoplethysmography (Finometer, Finapress; Netherlands). Heart rate was monitored continuously by ECG (Datex-Ohmeda; Helsinki, Finland).

With each trial, steady state ventilation at the desired oxygen saturation was maintained for an average of 4 minutes prior to the start of exercise (83). Subjects completed 3.5 minutes of dynamic exercise. Steady-state measures of parameters of interest (heart rate, blood pressure, oxygen saturation, blood velocity) were analyzed from the last 30 seconds of both rest and exercise, followed by a measure of arterial diameter. A
minimum of 10 minutes of normoxic rest separated each trial. A timeline can be found in Appendix A.

**Data Analysis and Statistics:**
All data are presented as mean±standard error and were analyzed using Minitab Version 16 (Mintab Inc.; State College, PA USA). All distributions of main outcome variables were approximately normal (p>0.05). Subject characteristics were compared via unpaired Student’s t-test. All resting measures are reported as an average of 2 trials within each condition (Normoxia %CV= 13±3, Hypoxia %CV=15±3). To account for higher perfusion pressures in adults with MS and to assess vasodilation, blood flow measurements were normalized for mean arterial blood pressure (FBF÷MAP) and are reported as vascular conductance (FVC; mL.min⁻¹.100 mmHg⁻¹).

**Aim 1:** The main dependent variable was FVC during normoxic exercise only. Hemodynamic variables from normoxic trials were analyzed using a general linear model repeated measures approach to determine the significance of the fixed effect of group (Control, MS) on parameters of interest. Bonferroni post hoc comparisons were performed when one-tailed significant effects were observed at a p≤0.05 level. The sample size for Aim 1 was determined a priori by the following power test equation (171): n=[2σ² · (Zα/2+Zβ)] · μd⁻², where α=0.05 and power(1-β)=0.80. Using previously published research in adults with hypertension (234), we set out to study a minimum of 8 subjects per group.
**Aim 2**: To determine the effect of hypoxia, a change in FVC ($\Delta$FVC) was calculated as: $\text{FVC}_{\text{exercise}} - \text{FVC}_{\text{rest normoxia}}$. The main dependent variable used to compare between groups was the relative change in $\Delta$FVC with hypoxia when compared to the respective normoxic exercise trial: $[\%\Delta\text{FVC} = (\Delta\text{FVC}_{\text{hypoxia}} - \Delta\text{FVC}_{\text{normoxia}}) + \Delta\text{FVC}_{\text{normoxia}} \times 100\%]$ (30). Hemodynamic variables were analyzed using a general linear model repeated measures approach to determine the significance of the fixed effect of group (Control, MS) and/or gas (Normoxia, Hypoxia) on parameters of interest. Bonferroni post hoc comparisons were performed when one-tailed significant effects were observed at a $p \leq 0.05$ level. In addition, covariate analysis was conducted post hoc using a general linear model approach to adjust for the potential effect of cardiovascular disease risk factors on primary outcome variables (FAV, HDL, cholesterol, glucose, triglycerides, BMI, waist circumference); this post hoc adjustment was conducted for discussion purposes only. The number of participants proposed (10 per group) was determined a priori using a power test equation with $\alpha=0.05$ and power=0.80 (171) and was based on published research looking at hypoxic vascular responses in older adults (30).
RESULTS

Subject Characteristics  Subject characteristics are summarized in Table 1. Eight adults with metabolic syndrome (MS) and thirteen healthy control subjects completed the current study (90% White non-Hispanic, 10% White Hispanic). Subjects were non-smokers and free from cardiovascular disease and sleep apnea, as determined by self-report. Six subjects (4 Control, 2 MS) were taking a daily vitamin supplement. All subjects exercised less than 3 hours a week (activities included walking, jogging, and/or biking), as determined by self-report.

There were no significant differences between groups in regard to age (29±2 Control, 29±3 MS, p=0.48). Adults with MS were clinically obese – displaying significantly higher weight, body mass index (BMI), and waist circumference—in addition to exhibiting greater blood pressure, fasting glucose, insulin, triglycerides, and lower HDL when compared with healthy controls (p<0.05). Specific MS criteria can be found in Appendix B. MS subjects had greater forearm volume and MVC when compared with healthy controls (p<0.01), however estimated lean forearm mass was similar between groups (Control 1005±55 vs MS 1097±57 g, p=0.13).

Aim 1: Exercise-Induced Skeletal Muscle Blood Flow in Human Metabolic Syndrome

Systemic Responses to Forearm Exercise  Results are summarized in Table 2. Mean arterial blood pressure was greater in MS adults when compared with healthy controls (Main effect of group, p<0.01) and values increased with exercise in both groups (Main
effect of exercise, p<0.01). Heart rate, oxygen saturation and end-tidal carbon dioxide levels were similar between groups (Main effect of group, p>0.05). Breathing frequencies were higher in adults with MS (Main effect of group, p=0.02), but tidal volumes were not significantly different between groups (Main effect of group, p=10); this relationship resulted in higher minute ventilation in adults with MS when compared with healthy controls (Main effect of group, p<0.01).

**Forearm Blood Flow Responses to Exercise**  Forearm blood flow (FBF) and vascular conductance (FVC) responses to dynamic exercise are summarized in Table 3 and Figure 1. There was a main effect of exercise intensity on FVC, with FVC increasing with increasing exercise intensity (p<0.01). Contrary to our hypothesis, measures of FVC at steady-state exercise as well as the rise in FVC from rest (ΔFVC) were both greater in MS adults when compared with healthy controls (Main effect of group, p<0.05; Figure 1). Reporting results as FBF provided comparable results (Table 3).

**Estimated Forearm Oxygen Delivery, Extraction and Consumption during Exercise**  Results are summarized in Table 4 and Figure 2. There was a main effect of exercise on P_vO_2, P_vCO_2, and venous pH that was not group-specific; P_vO_2 and venous pH decreased while P_vCO_2 increased from rest to steady-state exercise at both exercise intensities (p<0.05). Oxygen content, a-v O_2 difference and oxygen consumption were estimated. Oxygen consumption increased with increasing exercise intensity (Main effect of exercise, p<0.01). Group differences in a-vO_2 difference were not detected (Main effect of group, p=0.33), however adults with MS exhibited greater total forearm
oxygen consumption and rise in oxygen consumption with exercise ($\Delta VO_2$) when compared with healthy controls (Main effect of group, $p<0.01$; Table 4, Figure 2). These differences were likely due to differences in FBF, given the ratio between the rise in FBF and VO$_2$ with exercise ($\Delta FBF/\Delta VO_2$) was similar between groups (Main effect of group, $p=0.37$; Figure 2).

**Aim 2: Hypoxia-Mediated Vasodilation in Human Metabolic Syndrome**

**Systemic Responses to Hypoxia:** Results are summarized in Table 2. Heart rates increased with hypoxia (~15 beat/min) similarly between groups (Main effect of hypoxia, $p<0.01$; Interaction of group and hypoxia, $p=0.35$). Oxygen saturation and end-tidal carbon dioxide levels both decreased with hypoxia (Main effect of hypoxia, $p<0.05$) and values were not significantly different between groups (Main effect of group, $p>0.05$). Minute ventilation increased with hypoxia (Main effect of hypoxia, $p<0.01$) although absolute values were higher in MS adults when compared with healthy controls (Main effect of group, $p<0.01$).

**Vascular Responses to Hypoxia** Forearm vascular responses to hypoxia are summarized in Table 2 and Figures 3-4. Despite higher absolute FVC in adults with MS, a hypoxia-mediated vasodilatory response ($\Delta FVC$) was observed during exercise that was not group-specific (Main effect of hypoxia, $p=0.04$; Interaction of group and hypoxia, $p=0.45$; Figure 3). Conclusions were maintained when the effect of hypoxia was analyzed between groups as an absolute value (FVC, Interaction of group and hypoxia, $p=0.46$; Table 3), a relative change from normoxia ($\%\Delta FVC$, Main effect of
group, p=0.30; Figure 4), and a slope of the relationship between FVC and arterial oxygen saturation (Main effect of group, p=0.44; Figure 4). These findings were contrary to our hypothesis.

Estimated Forearm Oxygen Delivery, Extraction and Consumption with Hypoxia

Results are summarized in Table 4 and Figure 5. During hypoxia, the partial pressure of oxygen in venous blood decreased compared with normoxic trials (Main effect of hypoxia, p<0.01). In addition, the partial pressure of carbon dioxide in venous blood decreased (Main effect of hypoxia, p=0.02) and venous pH increased (Main effect of hypoxia p<0.01). These responses were not significantly different between groups (Main effect of group, p>0.05). Despite lower levels of inspired oxygen and arterial oxygen content, forearm oxygen consumption was maintained at normoxic levels in both groups across workloads (Main effect of hypoxia, p=0.08). This maintained VO₂ was likely due to a combination of increased forearm blood flow and reduced venous oxygen content (Main effect of hypoxia, p<0.05). The ratio of the rise in FBF and VO₂ (ΔFBF₂/ΔVO₂) was increased from normoxic levels, and a difference between groups was not detected (Main effect of hypoxia, p<0.01; Main effect of group, p=0.07).
DISCUSSION

The current study measured forearm vascular responses to exercise under both normoxic and hypoxic conditions to explore two individual aims: Aim 1) Determine whether adult humans with MS exhibit blunted exercise-induced skeletal muscle blood flow, Aim 2) Determine whether blood flow responses to exercise in MS are attenuated with additional physiological stress. We hypothesized forearm exercise muscle blood flow would be lower in adults with MS and the rise in exercise blood flow due to hypoxia would be blunted when compared with responses in healthy controls. In contrast to our hypotheses, novel findings from this study indicate: 1) Forearm blood flow during exercise at absolute workloads is increased in adults with MS when compared with healthy control subjects, 2) Hypoxia-mediated vasodilation is preserved in adults with MS during forearm exercise at absolute workloads when compared with healthy control subjects.

Aim1: Exercise-Induced Skeletal Muscle Blood Flow in Human Metabolic Syndrome

With exercise onset, blood flow increases to match oxygen delivery with skeletal muscle metabolic rate (9, 104, 192, 224). However, animal models of MS are known to exhibit impaired functional vasodilation in response to simulated exercise (87, 90, 92, 115, 166, 202, 248-250). Reduced functional vasodilation can lead to increased vascular resistance, impaired oxygen delivery, metabolic waste removal, and glucose disposal – all of which may be especially detrimental in this population and may result in earlier fatigue, reduced exercise tolerance, and aversion to physical activity. Despite novel
findings in animal models, it was previously unknown whether adult humans with MS exhibit muscle blood flow limitations during dynamic exercise.

Contrary to our hypothesis, results from the current study suggest steady-state forearm exercise hyperemia is not reduced in human MS. Thus, any impairment(s) in vascular control shown to exist in other models of MS are not seen when total blood flow to the human forearm is assessed during moderate, absolute-intensity exercise. These findings confirm those shown previously in otherwise healthy obese humans (102, 152, 168, 181, 208) and extend results to human MS. Interestingly, vascular responses to forearm exercise were not only maintained, but increased in human MS when compared with healthy controls; this may be due to: 1) larger forearm sizes, 2) altered adipose tissue blood flow, 3) impaired blood flow distribution, 4) altered metabolic need.

Adults with MS in the current study exhibited significantly larger total forearm sizes (FAV) when compared with healthy, lean controls (Table 1). Previous research in obese adults suggests this increase in forearm size is primarily due to an increase in adipose tissue rather than skin or lean mass (12) and this is supported in the current study (Table 1; group differences in estimated lean mass are not detected, p=0.13). Increased blood flow to adipose tissue might explain differences in whole limb flows, but available research suggests the overall contribution of adipose tissue to total limb flow is relatively small (12), the majority of exercise-induced blood flow is confined to the working muscle (111), and adipose tissue blood flow is lower in obese adults, adults with type 2 diabetes, and animal models of MS when compared with healthy controls.
Additionally, when the vasodilatory response to exercise ($\Delta$FVC from rest) was assessed (minimizing the potential contribution of metabolically inactive tissues to exercise hyperemia) conclusions were maintained (Figure 1). Taken together, it is unlikely adipose tissue blood flow can completely explain the observed group differences in forearm blood flow in the current study.

Increased exercise blood flow in human MS may be the result of altered blood flow distribution within a given muscle bed. With exercise onset, blood flow increases to those muscle fibers that are metabolically active; however, recent research in the obese zucker rat (OZR) uncovered extensive perfusion-demand mismatch resulting in higher flow dispersion in OZR when compared with control animals (91, 95, 247). Impaired distribution, or increased blood flow to inactive tissues, may require increased total limb flow in order to meet oxygen needs of metabolically active skeletal muscle fibers. Higher whole limb blood flows in some animal models of MS support this concept (7, 239). On the other hand, altered distribution may be compensated for by increased oxygen extraction in the working muscle; thus, despite perfusion-demand mismatch, measures of whole limb oxygen consumption in MS subjects would be expected to mirror that observed in healthy controls.

The above theory of maldistribution is based on the assumption that oxygen consumption needs are identical between groups. However, it is possible adults with MS exhibit increased metabolic need at the same absolute workload. Higher oxygen consumption at a given absolute workload has been observed previously in obesity,
although most research is limited to whole body exercises (such as cycling or treadmill walking) where increased mass of limb segments and/or increased work of breathing may be responsible for higher metabolic need (52, 139). In attempt to address the possibility of greater metabolic need during non-weight bearing exercise in human MS, we estimated forearm oxygen consumption in the current study. Results are presented in Table 4, Figure 2, and are discussed in more detail below.

The aim of this research was to determine vascular responses to forearm exercise at the same absolute exercise intensities (8 and 12 kg) and contraction duration (20 contractions/min at a duty cycle of 1 second contraction : 2 seconds relaxation) between groups. This approach was designed based on the following research revealing: 1) similar oxygen consumption between young and older adults at the same absolute exercise intensities (62, 245) and 2) an important role for contraction duration on oxygen consumption during exercise (104, 187). Given tightly controlled exercise conditions (same absolute workload and contraction duration), we were surprised to observe significantly higher blood flows in our cohort of MS adults when compared with healthy controls. Considering exercise muscle blood flow is thought to be more closely related to metabolic cost (VO₂) than contractile work (9, 104, 187, 192, 224), it is reasonable to propose higher flows in adults with MS may be due to a greater metabolic cost of exercise at the same absolute intensity.

Consistent with this concept, Sanderson et al (1996) have shown despite similar muscle tensions during simulated exercise, animals models of MS exhibit greater total ATP
synthesis when compared with healthy controls (197). Thus, when completing the same amount of work, MS animals had a higher demand for ATP, resulting in increased mitochondrial activation and an increased rate of ATP synthesis per unit of work (197). Results from the current study extend this idea to human MS; adults with MS were shown to exhibit higher levels of oxygen consumption despite similar absolute exercise intensities, maximal voluntary contraction (MVC), and amount of lean tissue – suggesting higher total metabolic cost of exercise (Table 4).

To examine the relationship between the rise in blood flow and metabolic need, we calculated the ratio of the change in FBF and VO₂ between groups at the same absolute workloads (ΔFBF/ΔVO₂) (187). Our results suggest whereas total VO₂ may be higher in MS adults, the increase in FBF is appropriate to meet metabolic demand (Figure 2). However, these findings do not explain the need for a higher VO₂ at the same absolute workload in human MS and we can only speculate why such differences in oxygen consumption were observed between cohorts in the current study. It is possible adults with MS exhibit increased non-contractile ATP consumption, altered skeletal muscle fiber type, and/or altered motor unit recruitment when compared with healthy controls (23, 77, 110). Consistent with this concept, Copp et al (2010) observed similar total hindlimb blood flow during exercise in diabetic animals when compared with healthy controls; however, blood flow to type II fibers was increased with diabetes (42). Future research will be necessary to explore these possibilities in human MS.
It is important to note hemoglobin levels were not directly assessed with the current design, therefore arterial and venous oxygen content were estimated using standard hemoglobin levels for all participants (14 g/dL). Given hemoglobin levels typically increase with increasing number of MS components [Hemoglobin 13.3±1.5 vs 14.0±1.3 g/dL, p<0.01; (155)], we may have underestimated group differences in total oxygen consumption (~2 mL/min or 5% of total oxygen consumption). Considering: 1) a close relationship exists between muscle blood flow and metabolic cost (VO₂), 2) muscle blood flow was higher in MS subjects when compared with controls, and 3) the potential for underestimation (rather than overestimation) of group differences in oxygen consumption, we are confident in our conclusion that adults with MS exhibit increased metabolic need at the same absolute workload when compared with healthy controls.

Taken together, results from the current study show exercise blood flow to be increased in human MS when compared with healthy controls. This finding is intriguing, given the depth of animal literature exposing a multitude of impaired vascular control mechanisms in MS. However, there are some important factors to consider when applying current results. First, a strong body of literature exists supporting limb-specific vascular control, thus results cannot be extrapolated to other skeletal muscle vascular beds (61, 170, 177). Second, whereas exercise blood flow and oxygen consumption are matched a lower exercise intensities, higher absolute blood flow at a lower workload may lead to an impaired ability to increase blood flow in proportion to need with greater physiological stress (a potential “ceiling effect”). This has been observed in animal models of MS, possibly due to structural remodeling of the microvasculature (85, 88, 146). Last, it is
possible altered vascular control mechanisms occur in human MS that were not observed in the current design when whole-limb flows were assessed. Along these lines, research in aging suggests altered vascular control mechanisms are present to maintain exercise blood flow, however impairments are uncovered with greater physiological stress as compensatory mechanisms become limited (28, 133, 178).

**Aim 1: Conclusion**

Research in animal models of MS have uncovered a magnitude of altered vascular control mechanisms leading to impaired functional vasodilation; whether these findings translate to human MS was previously unexplored. We hypothesized vascular responses to forearm exercise at absolute workloads would be reduced in human MS. Contrary to our hypothesis, functional vasodilation to the whole limb was increased in MS adults at absolute intensities. This increase in FVC with MS may be due to increased adipose tissue blood flow, altered flow distribution, and/or increased metabolic demand. Data from the current study support the concept of increased metabolic need at the same absolute workload in human MS when compared with healthy controls. This increased metabolic demand likely explains increased blood flow during exercise in this population. Future work will be necessary to elucidate the physiological need for such an adaptation in human MS, the mechanisms behind such changes, and the effect such changes have on vascular responses under conditions of greater metabolic need (i.e. higher exercise intensities and/or enhanced physiological stress).
Aim 2: Hypoxia-Mediated Vasodilation in Human Metabolic Syndrome

Systemic hypoxia introduces a physiological stimulus that requires a unique balance between sympathetically-mediated constriction and compensatory vasodilation to maintain oxygen delivery and meet metabolic demand (28, 59, 194, 213, 240, 241). The specific vascular control mechanisms responsible for local skeletal muscle blood flow under normoxic conditions appear to be both enhanced and altered with hypoxia (27). Thus, despite maintained steady-state normoxic exercise blood flow in human MS, an imbalance between vasodilatory and vasoconstrictor mechanisms may manifest with enhanced physiological stress. Along these lines, disorders shown to increase the risk of developing cardiovascular disease (such as obstructive sleep apnea and type 2 diabetes) appear to complicate the acute vascular response to systemic hypoxia in humans at rest (10, 53, 164, 185, 236).

Given previous research supporting altered vascular control mechanisms and impaired hypoxia-mediated vasodilation in animal models of MS (94, 97), we hypothesized adults with MS would exhibit blunted vascular responses to hypoxia during exercise when compared with healthy control subjects. Despite tightly controlled experimental conditions, hypoxia-mediated vasodilation during exercise was preserved in adults with MS during forearm exercise when compared with healthy control subjects. Conclusions were maintained when results were expressed in both absolute (FVC, ∆FVC) and relative (%∆FVC) terms. Given previous research in animal models of MS supporting altered vascular control, the lack of detectable difference between group responses to hypoxia during exercise may be due in part to compensatory vascular control
mechanisms. In healthy animals, hypoxia-mediated vasodilation is dependent upon combined nitric oxide (NO) and prostaglandin release from the vascular endothelium (94). However, dilation in the obese zucker rat (OZR) is predominantly prostaglandin-mediated, with a reduced role for NO (94); thus, the OZR relies on alternative vascular control mechanisms in attempt to compensate for any impairment in NO. Consistent with this concept, older adult humans exhibit preserved hypoxia-mediated vasodilation during low intensity forearm exercise, despite altered NO signaling (30). Thus, it is reasonable to speculate MS adults exhibit impaired vascular control but appropriately compensate via alternative mechanisms; however, specific vascular control mechanisms were not assessed with the current study design.

Whereas a difference in compensatory vasodilation between groups was not detected, a marked heterogeneity in FVC responses to hypoxia was observed. It appears there is no uniform pattern for hypoxia-mediated vasodilation within or between groups, despite strictly controlled exercise and hypoxic conditions. Our design did not directly test the role of sympathetic constriction versus metabolic dilation, although we speculate these differential responses to acute hypoxia depict the presence and balance of diverse vascular control mechanisms (Figure 4).

In a recent editorial, Charkoudian (32) emphasized variability in responses may be key to understanding integrative function. In support of this concept, hyperemic responses to hypoxia in obese patients with obstructive sleep apnea (OSA) were shown to be variable (183, 185). Remsburg and colleagues (1999) observed two distinct patterns of
response to hypoxia in adults with OSA—one-half of adults behaved similarly to controls by reducing resistance to flow with hypoxia whereas the remaining patients increased resistance (185). These vasodilatory responses were unable to be explained by differences in weight, BMI, age, or hypertension when analyzed post hoc (185). Similarly, the lack of detectable group differences in %ΔFVC in the current study could not be explained when results were statistically adjusted for FAV, HDL cholesterol, glucose, triglycerides, BMI, or waist circumference (Effect of group: p=0.31 before adjustment, p=0.26 after adjustment). Future studies are necessary to elucidate the role individual differences play during hypoxic exercise.

Physiological variability provides rationale for future studies to assess specific factors that may play a role in vascular responses to hypoxia, however it also brings into question whether the current study design was powered to detect differences. The number of participants proposed (10 per group) was determined by a power test equation using group means and standard deviations for the main outcome variable (%ΔFVC) from research recently published in young and older adults (30). The current study included 13 healthy controls but only 8 adults with MS. Due to observed heterogeneity in vascular responses to hypoxia in the current cohort, post hoc power analysis was completed. Results identified a minimum of 200 subjects per group would be necessary to detect group differences in the main outcome variable. Therefore, it is unlikely a Type II error occurred nor that a detectable difference in whole limb blood flow responses to hypoxia during exercise exists between healthy controls and adults with MS.
The primary focus of the present study was to examine vascular responses to hypoxia during exercise in human MS. Whereas group differences in compensatory vasodilation were not detected, the present investigation uncovered increased metabolic need at the same absolute exercise intensity in MS subjects. Taking this into consideration, it is possible the "maintained" level of vasodilation observed may be limited when compared with the increase in metabolic demand and concentration of local metabolites (i.e. adenosine, lactate, etc). To explore this possibility, we examined the ratio between the rise in FBF and VO$_2$ with exercise under normoxic and hypoxic conditions (Figure 5). A difference between groups was not detected, however there was a trend for reduced matching in human MS when compared with responses in healthy controls (Main effect of group, p=0.07; Interaction of group and hypoxia, p=0.14). This observation is intriguing. Whereas vascular responses appear to match metabolic need under normoxic conditions, it is reasonable to propose “maintained” compensatory vasodilation may not result in a matching between forearm blood flow and oxygen consumption under hypoxic conditions in all subjects. From a clinical perspective, hypoxic stimuli translate to activities such as air travel [S$_a$O$_2$ range 85-95%; (119)], exposure to high elevations [4000 m, S$_a$O$_2$ ~84%;(135)], and sleep disordered breathing, in addition to events resulting in local ischemia such as stroke and myocardial infarction. The mechanisms behind increased metabolic need at the same absolute workload in MS remain to be resolved; future work will be necessary to understand this relationship under conditions of greater physiological stress and its clinical implications.
Aim 2: Conclusion

Hypoxia provides an enhanced metabolic signal relative to exercise alone that challenges oxygen delivery and alters sympathetic tone. Research in animal models of MS exhibit impaired hypoxia-mediated vasodilation; whether these findings translate to human MS was previously unexplored. We hypothesized vascular responses to hypoxia during forearm exercise would be reduced in human MS. Contrary to our hypothesis, hypoxia-mediated vasodilation was maintained in human MS. Despite tightly controlled experimental conditions, vascular responses were heterogeneous between and within groups. In addition, the observed functional vasodilation in human MS, whereas matched with control subjects, may be impaired when compared with increased metabolic need. Future work will be necessary to elucidate specific vascular control mechanisms responsible for such heterogeneity in vascular control.

Experimental Considerations

A potential limitation of the present study is the slightly lower (0.5-0.7% or ~6 Torr) end-tidal carbon dioxide (CO₂) levels that occurred during hypoxic trials. Considering, 1) changes in CO₂ with hypoxia were not different between groups (Table 2), and 2) a relationship between changes in CO₂ and hypoxia-mediated vascular responses was not observed in the current study (Appendix C3), we feel this is a minor limitation. Consistent with this observation, CO₂ levels have been shown to have little (3) to no (183) effect on peripheral blood flow. In addition, Heistad & Wheeler (1970) defined reduced oxygen saturation as the primary mechanism for peripheral vasodilation during acute hypoxia, regardless of CO₂ levels (112). Taken together, any differences in CO₂
levels that occurred with hypoxia in the present investigation do not impact conclusions regarding hypoxia-mediated vasodilation.

Hypoxia is a non-specific physiological stimulus that relies on the peripheral chemoreflex to increase sympathetic nerve activity. Any differences in chemoreflex sensitivity could alter the rise in sympathetic outflow between groups and the balance between vasodilatory and vasoconstrictive mechanisms. Whereas research is not available in human MS, obese adults are known to exhibit preserved peripheral chemoreflex activation with hypoxia (167). Narkiewicz et al (1999) observed similar ventilatory and sympathetic responses to hypoxia between groups (167). Sympathetic nerve activity was not directly measured in the current study, however increases in heart rate and minute ventilation in response to hypoxia/exercise were not significantly different between groups (Table 2). In addition, research suggests changes in plasma catecholamines in response to a hypoxic stimulus are similar in diabetics when compared with healthy controls (236). Taken together, any differences in hypoxic responses between groups in the current study are likely local in nature; however, future research will be necessary to directly assess chemoreflex sensitivity and resultant changes in muscle sympathetic nerve activity in adults with MS.

**Interesting Findings**

Whereas not an aim of the current project, adults with MS exhibited increased hypoxic-vasodilatory responses when compared to healthy controls at rest (Appendix C4). Adults with MS are known to exhibit heightened sympathetic nerve activity that is well correlated with combined obesity and hypertension (72, 140, 141, 214). We speculate
increased hypoxic vasodilation at rest may be achieved by offsetting greater sympathetic constriction with metabolic dilation, possibly due to an altered metabolic milieu. It is also possible adults with MS exhibit a blunted sympathetic vasoconstrictor response to hypoxia at rest. Consistent with this concept, healthy older adults are known to exhibit heightened sympathetic nerve activity combined with blunted adrenergic responsiveness to sympathetic activation at rest (55, 56, 60, 61). If this is the case in MS, blunted adrenergic responsiveness may explain the increase in hypoxia-mediated vasodilation at rest; however future studies will be necessary to elucidate potential mechanisms.

**Conclusion**

We have shown for the first time that forearm blood flow responses to exercise are increased in human MS. Our findings may be the result of increased adipose tissue blood flow and/or impaired flow distribution, however group differences are most likely the result of increased metabolic need at the same absolute workloads in MS. The mechanisms behind increased oxygen consumption at the same absolute workload in human MS are largely unexplored. The present investigation also observed maintained hypoxic compensatory vasodilation during exercise in MS adults when compared with healthy controls. Vascular responses to hypoxia were highly variable both within and between groups. Such heterogeneous responses were likely due to individual-specific relationships between sympathetically-mediated vasoconstriction and local vasodilation. Taken together, results from the current study contradict research from animal models
of MS; thus the ability to translate such findings from animals to humans is apparently limited.
ACKNOWLEDGEMENTS

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Thank you to the authors for their individual contributions. TDE: data collection, analysis, and manuscript preparation. GMB: data collection and manuscript preparation. DFP: design, data collection and analysis. JRD: subject recruiting, scheduling and data collection. MWE, JJS, LTP: placement of IVs, medical supervision, and data collection. WGS: design, data collection, manuscript preparation.

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### Table 1.1: Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>Control (n=13)</th>
<th>Metabolic Syndrome (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>9/4</td>
<td>7/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29±2</td>
<td>29±3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74±3</td>
<td>127±8*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24±1</td>
<td>39±3*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>80±3</td>
<td>121±5*</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>76±4</td>
<td>88±4*</td>
</tr>
<tr>
<td>Insulin (uU/mL)</td>
<td>11±1</td>
<td>36±14</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>104±16</td>
<td>230±40*</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>63±5</td>
<td>38±7*</td>
</tr>
<tr>
<td>FAV (mL)</td>
<td>1011±50</td>
<td>1546±86*</td>
</tr>
<tr>
<td>Est. Lean Forearm mass (g)</td>
<td>1005±55</td>
<td>1097±57</td>
</tr>
<tr>
<td>MVC (kg)</td>
<td>41±2</td>
<td>47±2*</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SE. Control n=13, MS n=8 unless otherwise noted (Waist: Control n=12, MS n=7; Insulin: Control n=5, MS n=5); *p<0.05 vs Control

BMI: Body Mass Index, HDL: High-density Lipoprotein, FAV: Forearm Volume, MVC: Maximal Voluntary Contraction, Est.: Estimated from DXA scans and FAV in larger population of Control and MS participants (Appendix C1).
Table 1.2: Systemic responses to exercise and hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Rest 8 kg</th>
<th>12 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (beat/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67±2</td>
<td>73±2</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>71±3</td>
<td>74±4</td>
</tr>
<tr>
<td>Normoxia</td>
<td>73±3</td>
<td>76±4</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>87±2†</td>
<td>88±5†</td>
</tr>
<tr>
<td><strong>Mean arterial blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92±1</td>
<td>95±1²</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>101±2</td>
<td>104±2¹</td>
</tr>
<tr>
<td>Normoxia</td>
<td>97±1²</td>
<td>104±1²</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>107±2²</td>
<td>108±2²ab</td>
</tr>
<tr>
<td><strong>SₚO₂ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>99±0.2</td>
<td>99±0.3</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>97±0.6§</td>
<td>97±0.4§</td>
</tr>
<tr>
<td>Normoxia</td>
<td>99±0.4</td>
<td>97±0.5§</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>80±0.6†</td>
<td>79±0.8†</td>
</tr>
<tr>
<td><strong>Ventilation (BTPS) (L/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.1±0.4</td>
<td>14.7±0.8</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>13.4±0.8</td>
<td>18.1±1.4</td>
</tr>
<tr>
<td>Normoxia</td>
<td>11.8±0.9a</td>
<td>15.2±0.8a</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>13.7±1.1aT</td>
<td>18.9±1.2aT</td>
</tr>
<tr>
<td><strong>Tidal volume (L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.8±0.1</td>
<td>0.9±0.1†</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>0.8±0.2</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Normoxia</td>
<td>1.0±0.2†</td>
<td>1.1±0.1†</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>0.9±0.1</td>
<td>1.0±0.1T</td>
</tr>
<tr>
<td><strong>Breathing frequency (breaths/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16±2</td>
<td>17±2</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>19±2</td>
<td>18±2</td>
</tr>
<tr>
<td>Normoxia</td>
<td>15±2</td>
<td>20±2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>18±2</td>
<td>20±2</td>
</tr>
<tr>
<td><strong>End-tidal CO₂ (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.7±0.2</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>5.7±0.2</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>Normoxia</td>
<td>5.7±0.2</td>
<td>5.8±0.3</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5.2±0.2†</td>
<td>5.0±0.2†</td>
</tr>
</tbody>
</table>

**Table 1.2: Systemic responses to exercise and hypoxia**

Data are presented as Mean ± SE. Control n=13, MS n=8 unless otherwise noted

(Ventilation, Tidal volume, Breathing frequency, End-tidal CO₂: Control n=9, MS n=6)

Main effect of group (* p<0.05 vs Control); Main effect of workload (a p<0.05 vs Rest, b p<0.05 vs 8 kg); Main effect of hypoxia († p<0.05 vs Normoxia); Interaction effect of group and hypoxia (§ p<0.05 vs Control Normoxia)
<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>8 kg</th>
<th>12 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoxia</td>
<td>Hypoxia</td>
<td>Normoxia</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.43±0.02</td>
<td>0.43±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Metabolic Syndrome *</td>
<td>0.47±0.02</td>
<td>0.48±0.01</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>Blood Flow (FBF, mL/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>75±8</td>
<td>77±11†</td>
<td>290±24(^a)</td>
</tr>
<tr>
<td>Metabolic Syndrome *</td>
<td>114±16</td>
<td>179±21†</td>
<td>420±38(^a)</td>
</tr>
<tr>
<td>Conductance (FVC, mL/min*100mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>82±9</td>
<td>82±11†</td>
<td>306±24(^a)</td>
</tr>
<tr>
<td>Metabolic Syndrome *</td>
<td>115±16</td>
<td>179±22†</td>
<td>407±40(^a)</td>
</tr>
</tbody>
</table>

**Table 1.3: Blood flow responses to exercise and hypoxia**

Data are presented as Mean ± SE. Control n=13, MS n=8.

Main effect of group (* p<0.05 vs Control); Main effect of workload (\(^a\) p<0.05 vs Rest, \(^b\) p<0.05 vs 8 kg); Main effect of hypoxia († p<0.05 vs Normoxia)
Table 1.4: Venous blood gases and estimated oxygen consumption in response to exercise and hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Hypoxia</th>
<th>8 kg</th>
<th>Hypoxia</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P\textsubscript{O}\textsubscript{2} (Torr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37±2</td>
<td>29±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>28±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21±1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>28±1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23±1&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>42±4</td>
<td>30±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>28±3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23±1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>28±1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23±1&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>P\textsubscript{CO}\textsubscript{2} (Torr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>41±2</td>
<td>39±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>52±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44±3&lt;sup&gt;st&lt;/sup&gt;</td>
<td>47±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45±4&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>42±3</td>
<td>36±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>47±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43±3&lt;sup&gt;st&lt;/sup&gt;</td>
<td>53±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49±3&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Venous pH (units)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.38±0.01</td>
<td>7.41±0.01&lt;sup&gt;T&lt;/sup&gt;</td>
<td>7.32±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.36±0.01&lt;sup&gt;st&lt;/sup&gt;</td>
<td>7.30±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.32±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Metabolic Syndrome</td>
<td>7.37±0.01</td>
<td>7.39±0.01&lt;sup&gt;T&lt;/sup&gt;</td>
<td>7.32±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.35±0.01&lt;sup&gt;st&lt;/sup&gt;</td>
<td>7.29±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.32±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Est. arterial O\textsubscript{2} content (mL/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>187±1</td>
<td>152±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>188±1</td>
<td>147±2&lt;sup&gt;T&lt;/sup&gt;</td>
<td>187±1</td>
<td>151±1&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>185±1</td>
<td>151±1&lt;sup&gt;T&lt;/sup&gt;</td>
<td>185±1</td>
<td>152±1&lt;sup&gt;T&lt;/sup&gt;</td>
<td>185±1</td>
<td>152±1&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Est. venous O\textsubscript{2} content (mL/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>135±6</td>
<td>113±5&lt;sup&gt;T&lt;/sup&gt;</td>
<td>97±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74±3&lt;sup&gt;st&lt;/sup&gt;</td>
<td>98±4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>132±1</td>
<td>114±7&lt;sup&gt;T&lt;/sup&gt;</td>
<td>93±1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77±7&lt;sup&gt;st&lt;/sup&gt;</td>
<td>90±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74±6&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Est. a-vO\textsubscript{2} Difference (mL/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>52±7</td>
<td>39±5&lt;sup&gt;T&lt;/sup&gt;</td>
<td>91±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72±3&lt;sup&gt;st&lt;/sup&gt;</td>
<td>90±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73±4&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>53±14</td>
<td>37±7&lt;sup&gt;T&lt;/sup&gt;</td>
<td>93±11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75±8&lt;sup&gt;st&lt;/sup&gt;</td>
<td>95±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78±6&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Est. Oxygen Consumption (mL/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.7±0.3</td>
<td>2.9±0.4</td>
<td>26.5±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.6±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.6±4.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31.7±2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic Syndrome &lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.2±1.0</td>
<td>6.3±1.6</td>
<td>36.0±4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.1±4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.5±4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>42.5±3.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 1.4: Venous blood gases and estimated oxygen consumption in response to exercise and hypoxia

Data are presented as Mean ± SE. Control n=11, MS n=7.

Main effect of group (* p<0.05 vs Control); Main effect of workload (<sup>a</sup> p<0.05 vs Rest, <sup>b</sup> p<0.05 vs 8 kg); Main effect of hypoxia (<sup>†</sup> p<0.05 vs Normoxia)
Figure 1.1: (A) Absolute forearm vascular conductance (FVC) and (B) the rise in FVC from rest (∆ FVC) during exercise at absolute workloads in healthy controls and adults with metabolic syndrome.

Control n=13, MS n=8. FVC increased with increasing exercise intensity [Main effect of workload (\(a p<0.05 \text{ vs } \text{rest}, \ b p<0.05 \text{ vs } 8 \text{ kg}\)]. Measures of FVC at steady-state exercise (A) as well as the rise in FVC from rest (B) were both greater in MS adults when compared with healthy controls [Main effect of group (\(^* p<0.05 \text{ vs Control}\)].
Figure 1.2: (A) Forearm oxygen consumption (VO$_2$), (B) the rise in VO$_2$ from rest ($\Delta$VO$_2$), and (C) the ratio between the rise in FBF and VO$_2$ from rest ($\Delta$FBF/$\Delta$VO$_2$) during exercise at absolute workloads in healthy controls and adults with metabolic syndrome.

Control n=11, MS n=7. Oxygen consumption increased with increasing exercise intensity [Main effect of workload (a p<0.05 vs rest, b p<0.05 vs 8 kg). Adults with MS exhibited greater total forearm oxygen consumption (A) and the rise in oxygen consumption with exercise (B) when compared with healthy controls [Main effect of group (* p<0.05 vs Control)]. However, the ratio between the rise in FBF and VO$_2$ with exercise (C) was similar between groups.
Figure 1.3: Rise in FVC from normoxic rest (ΔFVC) during exercise at absolute workloads under normoxic and hypoxic conditions in healthy controls and adults with metabolic syndrome.

Control n=13, MS n=8. ΔFVC increased with increasing exercise intensity [Main effect of workload (b p<0.05 vs 8 kg)] and was greater in MS adults when compared with healthy controls across conditions [Main effect of group (* p<0.05 vs Control)]. A hypoxia-mediated vasodilatory response was observed during exercise [Main effect of hypoxia († p<0.05 Normoxia vs Hypoxia)] that was not group-specific [Interaction between group and hypoxia, p=0.46].
Figure 1.4: (A) Compensatory hypoxic vasodilation (\(\Delta FVC\)) and (B) the slope of the relationship between forearm vascular conductance (FVC) and arterial oxygen saturation (\(S_{\text{PO}_2}\)).

Control n=13, MS n=8. A hypoxia-mediated vasodilatory response was observed during exercise that was not group-specific. (A) Compensatory hypoxic vasodilation (\(\Delta FVC\)) was similar between groups [Main effect of group, \(p=0.30\)]. (B) Vascular responses to hypoxia were heterogeneous. A negative slope is indicative of hypoxia-mediated dilation and a positive slope is indicative of hypoxia-mediated constriction [Main effect of workload, \(^b\)\(p=0.05\); Main effect of group, \(p=0.44\)].
Figure 1.5: The ratio between the rise in FBF and VO₂ ($\Delta$FBF/$\Delta$VO₂) during exercise at absolute workloads under normoxic and hypoxic conditions in healthy controls and adults with metabolic syndrome.

Control n=11, MS n=7. The ratio of the rise in FBF and VO₂ with hypoxia was increased from normoxic levels [Main effect of hypoxia († p<0.05 Normoxia vs Hypoxia)] and a difference between groups was not detected [Main effect of group, p=0.07].
SYMPATHETIC CONTROL OF BLOOD FLOW DURING EXERCISE IN HUMAN METABOLIC SYNDROME

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ABSTRACT

Background: Animal models of metabolic syndrome (MS) exhibit increased sympathetic nerve activity, basal adrenergic tone, and α-adrenergic responsiveness; whether these findings translate to human MS was previously unexplored. We hypothesized humans with MS (n=14) would exhibit increased α-adrenergic responsiveness during exercise when compared with healthy controls (n=16). In addition, we hypothesized the level of vasoconstriction during exercise would be positively related to resting muscle sympathetic nerve activity (MSNA).

Methods: We measured forearm blood flow (BF, Doppler ultrasound) and MSNA (microneurography of the peroneal nerve) at rest and during dynamic forearm exercise (15% of maximal voluntary contraction, 20 contractions/min). A brachial artery catheter was inserted for measures of blood pressure (BP) and local administration of α-adrenergic agonists (phenylephrine, PE α₁ and clonidine, CL α₂). To account for higher BP in MS, we assessed changes in forearm vascular conductance (VC=BF÷BP).

Results: MSNA and forearm BF were greater in MS subjects when compared with healthy controls (Main effect of group, p<0.05). During exercise, vasoconstriction to PE was similar between groups (Main effect of group, p=0.11) and the level of PE-mediated vasoconstriction during exercise was inversely related to MSNA in MS adults (r=0.6, p=0.05). Adults with MS exhibited greater vasoconstriction to CL infusion when compared with healthy controls (Main effect of group, p<0.01); no relationships between MSNA and CL-mediated vasoconstriction were detected.

Discussion: α₁-adrenergic responsiveness is similar between MS subjects and healthy controls during exercise due, in part, to an inverse linear relationship between MSNA.
and $\alpha_1$-mediated vasoconstriction in MS subjects. In contrast, adults with MS exhibit increased $\alpha_2$-mediated vasoconstriction during exercise when compared with healthy controls that is not related to MSNA –suggesting other factors (i.e. endothelial dysfunction, reduced responsiveness to metabolites) may play a more direct role in the observed response.
INTRODUCTION

Adults with metabolic syndrome (MS) are obese, hypertensive, hyperglycemic, dyslipidemic, and at high risk of developing cardiovascular disease and type 2 diabetes (4, 26, 78-81). In addition, MS adults exhibit increased muscle sympathetic nerve activity (MSNA) when compared with healthy adults, which has been linked to increased rates of cardiovascular morbidity and mortality (73, 117, 141, 195). The exact mechanisms behind this increase are unknown; however a rise in MSNA has been attributed to changes in central autonomic regulation, a stiffening of receptive fields within the carotid bodies, and changes in body composition and/or insulin sensitivity (72, 124, 143, 195, 199, 206, 232, 233).

In response to sympathetic activation, nerve terminals primarily release norepinephrine, which binds $\alpha$-adrenergic receptors and mediates vascular smooth muscle contraction (13, 221). Chronic sympathetic activation—commonly observed in MS—may result in altered neurotransmitter release, receptor number, receptor affinity, and/or downstream signaling (50, 100, 101, 154, 163). Consistent with this concept, animal models of MS exhibit increased MSNA, in addition to increased basal $\alpha$-adrenergic tone and responsiveness when compared with healthy controls (25, 89, 166, 174, 211).

During exercise, a given amount of sympathetic activation has been shown to evoke less vasoconstriction in active muscle beds to optimize blood flow to metabolically active tissues (termed “functional sympatholysis”) when compared with responses at rest (148, 184, 192, 221). However, animal models of MS exhibit increased $\alpha$-
adrenergic responsiveness –or impaired functional sympatholysis – during simulated exercise (84, 87, 90); similar results have been reported in human aging and hypertension (60, 234). High MSNA and altered vasoconstrictor tone may limit the ability to augment muscle blood flow with increased metabolic demand, potentially resulting in impaired exercise blood flow, oxygen delivery, and/or blood flow distribution; such impairments have important implications for glucose uptake, blood pressure regulation, aerobic capacity, and exercise tolerance.

Given experimental evidence supporting altered neurovascular control in animal models of MS and the importance of the sympathetic nervous system to blood flow control and blood pressure regulation in humans, it is surprising more is not known regarding neural control of blood flow and its functional significance in human MS. This study aimed to determine whether adults with MS exhibit increased α-adrenergic responsiveness during exercise and whether α-adrenergic responsiveness during exercise is related to higher levels of MSNA commonly observed in this population. We hypothesized adults with MS would exhibit increased vasoconstrictor responses to intra-arterial infusion of α-adrenergic agonists during exercise and the degree of α-adrenergic vasoconstriction during exercise would be positively related to MSNA. To test these hypotheses, we measured forearm blood flow (Doppler ultrasound) during dynamic exercise in adults with MS and age-matched healthy controls. We assessed adrenergic responsiveness during exercise using intra-arterial infusion of α-adrenergic receptor agonists. In addition, MSNA (microneurography of the peroneal nerve) was measured to explore the direct link between increased sympathetic nerve activity and local blood flow control.
MATERIALS AND METHODS

Subjects

Two groups (MS, healthy controls) of relatively young (18-55 years) adult men and women were recruited from the University of Wisconsin – Madison campus and surrounding areas using Human Subjects IRB-approved advertisements. All subjects completed a screening process in which physical activity and personal health history (including history of medications and family history of cardiovascular disease; Appendix D) were assessed. All control subjects were lean (waist circumference ≤102 cm for males and ≤88 cm for females), normotensive (resting blood pressure <130/<85 mmHg), and otherwise healthy as determined by laboratory-measured blood lipid and glucose levels. All subjects were non-smokers, were free from overt cardiovascular disease and neurological disorders, and were not taking any cardiovascular or glucose/lipid-lowering medications, as determined by self-report. With regard to arterial catheter risks, subjects who reported general history of prolonged or difficult wound healing or any problems with bleeding or clotting were excluded.

Adults were characterized as having MS if, after in-person assessment, subjects met at least three of the following National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria as modified by the American Diabetes Association: central obesity (waist circumference >102 cm males, >88 cm females), pre-hypertension (resting blood pressure ≥130/≥85 mmHg), hypertriglyceridemia (triglycerides ≥150 mg.dL⁻¹), hyperglycemia (fasting glucose ≥100 mg.dL⁻¹) and/or dyslipidemia (HDL<40 mg.dL⁻¹ males, <50 mg.dL⁻¹ females) (4, 98).
Female subjects were premenopausal and women had a negative urine pregnancy test on each study day. To avoid confounding effects of changes in female hormone levels, all women were studied during the early follicular phase/placebo phase (days 1-6) of the menstrual cycle, as determined by self-report (33, 153, 162).

Subjects were instructed to refrain from exercise, non-steroidal anti-inflammatory drugs, alcohol, and caffeine for 24-hours prior to each study day. All studies were completed after a 10-hour fast. Written informed consent was obtained from all subjects prior to study procedures. All procedures were approved by the Institutional Review Board at the University of Wisconsin – Madison and conformed to the standards set by the Declaration of Helsinki. All testing was performed in the Bruno Balke Biodynamics Laboratory at the University of Wisconsin – Madison.

Descriptive Measurements

Body composition and central adiposity were determined by waist circumference, body mass index, and dual-energy x-ray absorptometry (DEXA; GE Lunar Prodigy; Milwaukee, WI). Lean forearm mass was analyzed from whole-body DEXA scans using anatomical landmarks (180). Forearm Maximal Voluntary Contraction (MVC) of the non-dominant arm was determined as the average of the two highest measurements from 5 trials using a hand dynamometer. The Paffenbarger physical activity questionnaire was completed to examine physical activity levels in a typical year (175). Subjects wore a pulse oximeter (Pulsox-300i; Konica Minolta, Osaka, Japan) on the index finger during two nights of sleep for oximetry and cardiac monitoring; portable
monitoring provided a measure of desaturation events and results were taken at face value (41).

After a 10-hour fast, whole blood was collected and levels of HDL cholesterol, triglycerides, and glucose were measured immediately (CardioChek; PTS Panels; Indianapolis, IN, USA). Additional blood samples were collected at rest and plasma was frozen at -80 degrees C to be analyzed at a later date. Given research suggesting MSNA levels may be driven by high plasma levels of insulin and/or leptin (72, 75, 124, 143, 195, 206), plasma insulin (radioimmunoassay; Wisconsin National Primate Research Center; Madison, WI), leptin (enzyme immunoassay; Wisconsin National Primate Research Center; Madison, WI), and catecholamine (norepinephrine, epinephrine; high-performance liquid chromatography, ARUP Laboratories; Salt Lake City, UT) concentrations were measured.

**Blood Flow Measurements**

Forearm blood flow (FBF) was measured using Doppler ultrasound (Vivid 7, General Electric; Milwaukee, WI). Blood flow was determined as the product of mean blood velocity (cm.s\(^{-1}\)) and vessel cross sectional area (\(\pi \times \text{radius}^2\) with radius measured in cm) and values were multiplied by 60 to convert from ml.s\(^{-1}\) to ml.min\(^{-1}\). A 12 MHz linear array probe was placed approximately midway between the antecubital and axillary regions, medial to the *biceps brachii* muscle, with a probe insonation angle of <60 degrees and the sample volume adjusted to cover the width of the artery using identical methods as described previously (200, 201, 241). The ultrasound probe operator
continuously adjusted the probe position to maintain a fixed insonation angle, compensating for subjects’ movements during exercise. Pulse-wave velocity was continuously assessed (beat-to-beat) and results were reported in 30-second intervals to reduce contraction-to-contraction-induced variability in blood flow.

A commercial interface unit (Multigon Industries; Younkers, NY) processed the angle-corrected, intensity-weighted Doppler audio information from the GE Vivid ultrasound system into a flow velocity signal sampled in real time with signal-processing software (PowerLab, ADinstruments; Colorado Springs, CO). All hemodynamic data were digitized, stored on a computer at 400 Hz, and analyzed off-line using PowerLab. Post-processing using PowerLab’s Chart application package yielded mean blood velocities.

Artery diameters were obtained from B-mode video images and measurements typically resulted in loss of pulse wave signal for 15 seconds. To determine vessel cross-sectional area, artery diameter was taken as the median of five measurements in late diastole, between muscle contractions. Arterial diameter was measured in the part of the artery running perpendicular to the ultrasound beam, identified by strong wall signals in a longitudinal section of the artery (measuring the distance between near and far intima-media interfaces). All measurements were made off-line by a trained operator. These procedures are identical to those in many laboratories (30, 46, 234).
Forearm Exercise

Each subject lay supine with the non-dominant arm extended to the side (approximately 90 degrees) at heart level. Dynamic and rhythmic, non-dominant forearm exercise required subjects squeeze and release two handles together 4-5 cm to raise and lower a weight over a pulley. Exercise was completed at a rate of 20 times per minute (at a duty cycle of 1 second contraction : 2 second relaxation) to the rhythm of a metronome. A mild workload (15% of MVC, ~6 kg) was used to minimize systemic effects of exercise (i.e. increases in MSNA, baroreflex activation, etc) (231). The majority of research regarding mechanisms of functional sympatholysis suggests sympatholytic factors are determined by relative exercise workloads (34, 36, 57, 107). This forearm exercise model is identical to that used in several laboratories (30, 46, 234) and resultant measures of vascular conductance have been shown to be repeatable across exercise trials spanning ~120 minutes (Appendix C5).

Intra-Arterial Pharmacological Intervention

Subjects lay supine for insertion of a brachial artery catheter (Arrow International Inc, Reading, PA, USA). Under aseptic conditions and after local anesthesia (2% lidocaine), a 20 gauge, 4.45 cm catheter was placed in the brachial artery of the non-dominant forearm in the antecubital fossa by an experienced physician. The catheter was used for local administration of vasoactive drugs and blood pressure monitoring.

All drugs were approved by the United States Food and Drug Administration for investigational use in this project under Investigational New Drug Application #110253.
All drugs were infused via the brachial artery catheter using a Twin Syringe Infusion pump (Pump 33, Harvard Apparatus; Holliston, MA, USA). Infusions were adjusted for lean forearm size and blood flow conditions to normalize concentrations of each drug between subjects/trials and to minimize systemic effects. The pump rate (mL.min⁻¹) for each drug infusion was calculated “on-the-fly” as: \[\text{Drug dose (ug.dL}^{-1}.\text{mL}^{-1}) \times \text{Lean forearm mass (mL)} \times \text{Steady-State blood flow (mL.min}^{-1})\] \div (100 \times \text{Drug Concentration (ug.min}^{-1}) \times \text{Resting blood flow (mL.min}^{-1})\] (60).

Selective α-adrenergic agonists were infused at rest and during exercise to evoke vasoconstriction and assess adrenergic responsiveness using previously published doses (61, 82, 153). Phenylephrine (Baxter Healthcare Corporation; Deerfield, IL, USA) is a selective α₁-adrenergic agonist and was infused at a rate of 0.03125 ug.dL⁻¹.min⁻¹. Clonidine (Anodyne Pharmaceuticals, Inc; Newport, KY, USA) is a selective α₂-adrenergic agonist and was infused at a rate of 0.15 ug.dL⁻¹.min⁻¹. In addition, a non-selective α-adrenergic antagonist (phentolamine) was infused during exercise in a subset of subjects (Control n=16, MS n=11; due to a drug shortage) to inhibit α-adrenergic vasoconstriction and test the effect of endogenous norepinephrine on adrenergic receptors (190, 220, 228, 237, 240). Phentolamine (Ben Venue Laboratories, Inc; Bedford, OH, USA) was infused for 6.5 minutes during steady-state exercise at a rate of 10 ug.dL⁻¹.min⁻¹, followed by a continuous maintenance dose (25 ug.min⁻¹).
Transient β-mediated vasodilation may occur during α-adrenergic blockade (222) and MS has been associated with reduced β-mediated vasodilation (150). To avoid potential confounding effects of differences in β-adrenergic regulation between groups, a continuous infusion of a non-selective β-adrenergic receptor antagonist (propranolol) was given throughout the study. Propranolol (Ben Venue Laboratories, Inc; Bedford, OH, USA) was given at rest as a loading dose (20 ug.dL⁻¹.min⁻¹ for 5 minutes) followed by a continuous maintenance dose (25 ug.min⁻¹) using previously published values (55, 134).

**Microneurography**

MSNA was assessed by microneurography in a subset of subjects (n=22; a clear recording could not be obtained from 4 subjects and 4 subjects declined participation in the procedure). With subjects in the supine position, multiunit, direct intraneural recordings of MSNA were obtained by percutaneous insertion of a unipolar tungsten microelectrode into muscle fascicles of the right peroneal nerve, posterior to the fibular head (33, 105, 252). Recording electrodes had a diameter of 200 um in the shaft, tapering to 1-5 um at the uninsulated tip (UNA32F2S; FHC, Bowdoin, ME, USA). A reference electrode was positioned subcutaneously approximately 4 cm from the recording electrode (UNR32FRS; FHC, Bowdoin, ME, USA). A muscle sympathetic fascicle was identified when taps on the muscle belly or passive muscle stretch evoked mechanoreceptive impulses and no afferent neural response was evoked by skin stimulation.
Neural signals were amplified (20,000 to 50,000 times), filtered (band-width 700-2000 Hz), rectified, and integrated (time constant, 0.1 second) to obtain mean voltage neurograms (Rys Systems; Milwaukee, WI, USA). Data were sampled in real time with signal-processing software (PowerLab, ADinstruments; Colorado Springs, CO) and analyzed off-line using PowerLab’s Chart application package. Sympathetic bursts in the integrated neurogram were identified using a custom-manufactured semi-automatic analysis program. Burst identification was controlled visually by a single investigator. Recordings were considered acceptable based on criteria discriminating MSNA from other neural signals (skin SNA, muscle spindle activity) and background noise (~3:1 signal to noise ratio). Nerve activity was quantified by determining the burst frequency (bursts per minute) and burst incidence (bursts per 100 heart beats) (105, 235).

**Protocol**

The study required one 90-minute screening visit, 2 nights of sleep-disordered breathing monitoring, and 2 study visits. All study visits began in the morning and were conducted at the same time within subjects. During the screening visit and after providing informed consent, subjects experienced anthropometric measures, blood sampling, and a DEXA scan.

On the first study day, subjects lay supine for the insertion of the arterial catheter. Over the next 3.5 hours, subjects experienced eight (8) individual trials. A timeline can be found in Appendix A. Blood pressure (indwelling arterial catheter; ICU Medical Inc, San Clemente, CA, USA), heart rate (ECG; Datex-Ohmeda; Helsinki, Finland), and brachial
artery blood velocity (Doppler ultrasound) were measured continuously. Arterial
diameter and steady-state measures of the aforementioned parameters were reported
from the last 30 seconds of rest/exercise/drug infusion. Trials were separated by a
minimum of 10 minutes of quiet rest and were conducted as follows:

- **Trial 1: Exercise Control:** After 2 minutes of quiet rest, subjects completed 7
  minutes of dynamic exercise at 15% of MVC.

- **Trial 2: β-adrenergic blockade:** After 2 minutes of quiet rest, subjects completed
  5 minutes of rest while receiving the loading dose of propranolol (20 ug.dL⁻¹.min⁻¹). After 5 minutes, the pump rate was reduced to achieve a continuous
  maintenance dose (25 ug.min⁻¹) for the remainder of the study.

- **Trials 3-6: α-adrenergic responsiveness:** After 2 minutes of quiet rest, subjects
  completed 7 minutes of dynamic exercise or rest; for the last 3 minutes of each
  trial, subjects received intra-arterial infusion of either phenylephrine (0.03125
  ug.dL⁻¹.min⁻¹) or clonidine (0.15 ug.dL⁻¹.min⁻¹). The trials were randomized,
  counterbalanced, and consisted of: Rest + Phenylephrine, Rest + Clonidine,
  15% MVC dynamic exercise + Phenylephrine, 15% MVC dynamic exercise +
  Clonidine.

- **Trial 7: Inhibition of α-adrenergic vasoconstriction:** After 2 minutes of quiet rest,
  subjects completed 10.5 minutes of dynamic exercise; for the last 6.5 minutes
  subjects received intra-arterial infusion of phentolamine (10 ug.dL⁻¹.min⁻¹). The
  pump rate was reduced to achieve a continuous maintenance dose (25 ug.min⁻¹)
  for the remainder of the study.
\begin{itemize}
  \item \textit{Trial 8: Verification of }\alpha\textit{-adrenergic blockade: }After 2 minutes of quiet rest, subjects completed 7 minutes of rest; for the last 3 minutes subjects received intra-arterial infusion of phenylephrine (0.03125 ug.dl\textsuperscript{-1}.min\textsuperscript{-1}).
\end{itemize}

Due to the long half-life of phentolamine, trials 7 and 8 were always conducted last (190, 220, 228, 237, 240). The final two (2) trials tested the effects of endogenous norepinephrine on adrenergic receptors; a control trial with phenylephrine infusion followed to confirm pharmacological inhibition of \(\alpha\)-adrenergic vasoconstriction with phentolamine (Appendix C6).

On the second study day, subjects lay supine for microneurography to collect measures of resting MSNA (Rys Systems; Milwaukee, WI). Study visits were separated by 2 days – 9 months. Blood pressure and heart rate were measured by electronic sphygmomanometer on the upper arm and ECG, respectively (Datex-Ohmeda; Helsinki, Finland). After achieving a clear nerve signal and 10 minutes of quiet rest, blood flow (Doppler ultrasound) and the above measures were assessed continuously during a 5-minute quiet resting trial. Subjects were instructed to avoid sympathoexcitatory maneuvers including Valsalvas and prolonged expirations and compliance was assessed using strain-gauge pneumography positioned at the midchest level (Madison, WI). MSNA values are reported as an average of three one-minute sections of data collected at rest (%CV = 10±2). MSNA was also measured during 7 minutes of dynamic forearm exercise at 15% MVC in a subset of subjects (n=21) to verify activity was maintained at resting levels (Appendix C7).
**Data Analysis and Statistics:**

All data are presented as mean±standard error and were analyzed using Minitab Version 16 (Mintab Inc.; State College, PA USA). All distributions for main outcome variables were approximately normal (p>0.05). Subject characteristics were compared via unpaired Student’s t-test. To account for potential individual differences in lean muscle mass and perfusion pressures, and to assess vasodilation, blood flow measurements were normalized for mean arterial blood pressure and forearm lean mass (FBF÷MAP÷lean mass) and are reported as lean vascular conductance (FVC; mL.min⁻¹.100 mmHg⁻¹.100g⁻¹).

**Aim 1:** The main dependent variable was the relative change in lean FVC with infusion of adrenergic agonists (%FVC). The percentage reduction (%FVC) with drug infusion was calculated as: \[
\frac{\text{FVC}_{\text{post infusion}} - \text{FVC}_{\text{pre infusion}}}{\text{FVC}_{\text{pre infusion}}} \times 100\%
\].

Hemodynamic variables were analyzed using repeated measures analysis of variance to determine the significance of the fixed effect of group (MS, Control) on parameters of interest. Bonferroni post hoc comparisons were performed when one-tailed significant effects were observed at a p≤0.05 level. In addition, covariate analysis was conducted post hoc using a general linear model approach to adjust for the potential effect of cardiovascular disease risk factors on primary outcome variables (waist circumference, glucose, HDL cholesterol, triglycerides); this post hoc adjustment was conducted for discussion purposes only. The number of participants (minimum of 11 per group) was determined *a priori* by a power test equation with α=0.05 and power=0.80 (171), using group differences from previously published research in older adults (60).
Aim 2: Linear regression analysis of covariance was used and Pearson’s correlation coefficients were calculated to assess the relationship between resting MSNA and α-adrenergic responsiveness (%FVC) (33, 168). Resting measures of MSNA were used for all comparisons based on previous research in healthy adults showing MSNA to remain at resting levels during dynamic forearm exercise at moderate intensities (<50% MVC) (231) in addition to unpublished observations from our lab (Appendix C7). The number of participants was determined a priori from previous research in healthy adults suggesting a minimum of 16 total subjects would provide 83% power to detect a correlation of 0.65 (108). Subjects were initially pooled (n=22) and assessed for significant effects, however no relationships between MSNA and adrenergic responsiveness were observed. Subjects were then separated by group for individual analysis (Control n=12, MS n=10) and one-tailed significant effects were discussed at a p≤0.05 level. Post hoc power analysis suggested n=13 would provide 80% power to detect a correlation of 0.65 (65).
RESULTS

Subject Characteristics  Subject characteristics are summarized in Table 1. Fourteen adults with metabolic syndrome (MS) and sixteen healthy control subjects participated in the current study (80% White non-Hispanic, 12% White Hispanic, 4% Asian, 4% African American). One subject reported diagnosed sleep apnea, however he presented with an average of 3 desaturation events per hour, as determined by 2 nights of pulse oximetry. Given a threshold of 15 desaturation events has been linked to abnormal apnea-hypopnea indices (169), he was included in the current study. Eight subjects (Control n=5, MS n=3) were taking a daily vitamin supplement. All subjects reported exercising less than 3 hours a week and when activity was assessed by questionnaire, the majority of participants reported physical activity ≤3000 kcal per week (Control n=12, MS n=14). All female subjects (Control n=5, MS n=5) reported having regular menses and were studied during the early follicular/placebo phase of the menstrual cycle (n=3 using hormonal birth control).

Groups were not significantly different in regard to age, lean forearm mass, and forearm MVC (p>0.05). On average, adults with MS were clinically obese – displaying significantly higher weight, body mass index (BMI), waist circumference, and body fat — in addition to exhibiting greater triglycerides, blood pressure, and lower HDL when compared with healthy controls (p<0.05). Adults with MS exhibited higher resting muscle sympathetic nerve activity (MSNA) when expressed as burst frequency and/or burst incidence (p<0.05). Specific MS criteria can be found in Appendix B.
Forearm Exercise Responses prior to β-adrenergic blockade

Results are summarized in Table 2 and Figure 1. Mean arterial blood pressure, heart rate, and brachial artery diameter were greater in MS adults when compared with healthy controls (Main effect of group, p<0.05) and values did not change with exercise (Main effect of exercise, p>0.05; Table 2). Forearm blood flow (FBF) was similar between groups at rest and both FBF and vascular conductance (FVC) increased with exercise. FBF and FVC during exercise were greater in MS subjects when compared with healthy controls (Main effect of group, p<0.05; Table 2). When normalized to lean muscle mass, there was a main effect of group on FBF and FVC, with MS subjects exhibiting higher values across resting and exercise conditions (p<0.05; Figure 1). These findings were supported when data were analyzed as a rise in lean FVC from rest to steady-state exercise (Main effect of group, p=0.05; ∆ lean FVC, Figure 1).

α₁-adrenergic responsiveness at rest and during exercise

Results are summarized in Table 3 and Figures 2-3 (Control n=16, MS n=13; 1 MS subject did not complete due to discomfort during initial phenylephrine infusion). Mean arterial blood pressure, heart rate, and brachial artery diameter were greater in MS adults when compared with healthy controls (Main effect of group, p<0.05) and values did not change with exercise (Main effect of exercise, p>0.05; Table 3). Forearm FBF and FVC were similar between groups at rest and increased with exercise, however exercise responses were greater in MS subjects when compared with healthy controls (Interaction effect of group and exercise, p<0.05).
Phenylephrine (α₁-adrenergic agonist) infusion resulted in a significant reduction in FBF and FVC at rest, and relative responses (% FBF, %FVC) were not different between groups (Interaction effect of group and condition, p>0.05; Figure 2, Appendix C8). An inverse linear relationship between resting α₁-adrenergic responsiveness and MSNA was observed in young healthy adults (r=0.5, p=0.04); adults with higher MSNA exhibited blunted α₁-vasoconstrictor responses. A relationship was not detected in MS subjects (r=0.3, p=0.22; Figure 3).

During exercise, phenylephrine-mediated vasoconstriction (%FVC) was blunted from resting levels and the level of constriction was similar between groups (Main effect of condition, p<0.01; Main effect of group, p=0.11; Figure 2). There was no observed relationship between MSNA and α₁-adrenergic responsiveness during exercise in healthy adults (r=0.1, p=0.39), however MS adults exhibited reduced vasoconstrictor responses with higher levels of MSNA (r=0.6, p=0.05; Figure 3).

α₂-adrenergic responsiveness at rest and during exercise

Results are summarized in Table 4 and Figures 4-5. Brachial artery diameter was similar between groups (Main effect of group, p=0.11) and values did not change with exercise (Main effect of exercise, p=0.39). Mean arterial blood pressure and heart rate were greater in MS adults when compared with healthy controls (Main effect of group, p<0.05) and values did not change with exercise (Main effect of exercise, p>0.05; Table 4). FBF and FVC were similar between groups at rest and increased with exercise;
values during exercise were greater in MS subjects when compared with healthy controls (Interaction effect of group and exercise, p<0.05; Table 4).

Clonidine ($\alpha_2$-adrenergic agonist) infusion resulted in a significant reduction in FBF and FVC at rest, with relative responses ($\%$FBF, $\%$FVC) greater in adults with MS when compared with healthy controls (Interaction effect of group and condition, p<0.01; Figure 4, Appendix C9). No relationship was observed between MSNA and clonidine-mediated vasoconstriction at rest in either group (Control $r=0.3$, p=0.22; MS $r=0.1$, p=0.37; Figure 5).

During exercise, clonidine-mediated vasoconstriction ($\%$FVC) was blunted from resting levels (Main effect of condition, p<0.01) and the level of constriction was greater in adults with MS (Interaction effect of group and condition, p<0.01; Figure 4). No relationships between MSNA and $\alpha_2$-adrenergic responsiveness were observed in either group during exercise (Control $r=0.3$, p=0.20; MS $r=0.0$, p=0.48; Figure 5).

**Sympathetic-mediated restraint of exercise blood flow**

Results are summarized in Table 5 and Figures 6-7 (Control n=16, MS n=11; 3 MS subjects did not complete due to a drug shortage). Brachial artery diameters were similar between groups and values did not change with exercise (Main effect of group, p=0.25; Main effect of exercise, p=0.13; Table 5). Heart rate was greater in MS adults when compared with healthy controls (Main effect of group, p<0.01) and heart rates increased from resting levels during exercise+phentolamine infusion (Main effect of
exercise, p<0.01; Table 5). Mean arterial blood pressure was greater in MS adults when compared with healthy controls (Main effect of group, p<0.01) and values did not change with exercise and/or drug infusion (Main effect of exercise, p=0.33; Table 5). FBF and FVC were greater in adults with MS (Main effect of group, p<0.05) and values increased with exercise (Main effect of exercise, p<0.05) (Table 5).

Phentolamine (α-adrenergic antagonist) infusion resulted in a significant increase in FBF and FVC during exercise, with relative responses (%FBF, %FVC) lower in MS adults when compared with healthy control subjects (Main effect of group, p<0.05, Table 5, Figure 6). To explore potential mechanisms behind this relationship, we correlated relative responses to phentolamine infusion during exercise with baseline MSNA (Control n=12, MS n=8). In healthy controls, MSNA was positively related to sympathetic restraint – subjects with high MSNA exhibited a greater relative increase in FVC with infusion of phentolamine (r=0.5, p=0.05). However, there was a trend for the inverse relationship in adults with MS (r=0.4, p=0.13); those with higher MSNA exhibited blunted sympathetic restraint (Figure 7).
DISCUSSION

The current study measured muscle sympathetic nerve activity (MSNA) and forearm blood flow during exercise and infusion of α-adrenergic agonists to explore two individual aims: 1) Determine whether adults with MS exhibit increased α-adrenergic responsiveness during exercise, 2) Determine whether α-adrenergic responsiveness during exercise is related to MSNA. We hypothesized adults with MS would exhibit increased α-adrenergic vasoconstrictor responses to intra-arterial infusion of adrenergic agonists and the degree of vasoconstriction would be positively related to MSNA.

Novel findings from this study indicate: 1) α₁-adrenergic responsiveness during exercise is not increased in adults with MS when compared with healthy controls, 2) α₁-adrenergic responsiveness during exercise is linearly and inversely related to MSNA in adults with MS, 3) Adults with MS exhibit increased α₂-adrenergic responsiveness during exercise when compared with healthy controls, 4) A relationship between α₂-adrenergic responsiveness and MSNA is not observed during exercise in either group. Taken together, results from the current study support altered neurovascular control in human MS that is receptor-specific.

α₁-adrenergic Receptor Responsiveness

Although data in humans are limited, α-adrenergic receptors are thought to be spatially distinct with α₁-adrenoceptors located primarily on large arterioles (5, 74, 125, 172, 173). Due to their proximal location and relative insensitivity to muscle metabolites, α₁-adrenergic receptors are thought to primarily regulate whole-muscle blood flow. Along
these lines, animal models of MS exhibit increased α₁-adrenergic responsiveness at rest and reduced whole-limb blood flow during simulated exercise that can be restored with α-adrenergic blockade (91, 95, 211). In contrast, results from the current study demonstrate maintained forearm blood flow and α₁-adrenergic responsiveness both at rest and during exercise in human MS when compared with healthy controls (Figures 1 and 2).

α₁-adrenergic Responsiveness at Rest
MSNA is highly variable between individuals, with values ranging 5- to 10-fold in young, healthy adults (33, 76, 108, 216). Initially, this variability led researchers to discount the ability to discern physiological relevance from measures of MSNA (229). However, it is now accepted that such variability is integral to cardiovascular control (123). Results from the current study (Figure 3) confirm recent findings in young healthy adults demonstrating an inverse relationship between MSNA and resting vasoconstrictor responses to phenylephrine infusion (33, 61); this physiological adaptation results in maintained blood flow despite potentially high levels of MSNA. The exact mechanisms behind this relationship are unknown, although research supports adrenergic receptor desensitization and/or receptor downregulation in response to chronic neural firing (14, 116, 238).

Although adults with MS exhibited maintained α₁-vasoconstrictor response to phenylephrine infusion, there was a trend (p=0.14) for increased responsiveness at rest (Figure 2). A post hoc power analysis identified a minimum of 55 subjects per group
would be necessary to detect group differences in %FVC; therefore we are confident in our conclusion of maintained $\alpha_1$-adrenergic responsiveness. However, the lack of a detectable relationship between MSNA and $\alpha_1$-adrenergic mediated vasoconstriction at rest suggests MS adults with higher MSNA do not exhibit the same receptor desensitization and/or downregulation that may occur in healthy control subjects (Figure 3). Consistent with this concept, Dincer et al (2006) demonstrated a lack of $\alpha_1$-adrenergic receptor down-regulation in a canine model of MS (54). Therefore, as MS progresses toward cardiovascular disease, we may observe increased $\alpha_1$-adrenergic responsiveness that could result in reduced whole-limb blood flow, altered glucose disposal, and/or impaired blood pressure regulation.

$\alpha_1$-adrenergic Responsiveness during Exercise

The vasoconstrictor responses to phenylephrine infusion observed at rest were attenuated during exercise in both groups (“functional sympatholysis”) and the level of sympatholysis was maintained in adults with MS when compared with healthy controls (Figure 2). To begin to understand specific mechanisms underlying whole-limb blood flow control during exercise in MS, we examined the relationship between $\alpha_1$-vasoconstrictor responses and MSNA. Whereas no relationship between MSNA and $\alpha_1$-mediated vasoconstriction was apparent in healthy control subjects, an inverse linear relationship in MS adults was observed; MS subjects with higher levels of MSNA exhibited reduced responsiveness to $\alpha_1$-adrenergic stimulation during forearm exercise when compared with lower MSNA levels (Figure 3). This adaptation may be important for maintaining bulk perfusion during exercise, considering exercise blood flow was
shown to be increased in this population (Figure 1). The specific mechanisms behind this adaptation are unknown. It is unlikely the observed relationship between $\alpha_1$-mediated vasoconstriction and MSNA is due to receptor desensitization or altered downstream signaling, given a similar relationship was not observed at rest.

Adults with MS exhibited enhanced MSNA when compared with healthy controls (Table 1). Prolonged $\alpha_1$-adrenergic receptor activation of the aorta can result in increased endothelial nitric oxide synthase (eNOS) expression (101). Under conditions of increased metabolic demand (i.e. exercise), an increase in eNOS expression may result in an increase in NO-mediated sympatholysis that is linearly related with MSNA. However, research suggests nitric oxide is responsible for <10% of functional sympatholysis in healthy adults (58) and animal models of MS demonstrate reduced NO bioavailability when compared with healthy controls (93, 94) Taken together, future research will be necessary to explore the potential for altered eNOS expression and its role in $\alpha_1$-mediated vasoconstriction during exercise in humans.

$\alpha_2$-adrenergic Receptor Responsiveness

In contrast to $\alpha_1$-adrenoceptors, $\alpha_2$-adrenergic receptors are thought to be located primarily on small, distal arterioles and control blood flow distribution within skeletal muscle vascular beds (5, 74, 125, 172, 173). $\alpha_2$-adrenergic receptors are more sensitive to muscle metabolites and play a more important role in functional sympatholysis when compared with $\alpha_1$-receptors (5, 74, 125, 172, 173). MS adults in the present investigation exhibited increased $\alpha_2$-adrenergic responsiveness at rest when
compared with healthy controls. The vasoconstrictor responses to clonidine infusion observed at rest were attenuated during exercise in both groups (“functional sympatholysis”), although the level of $\alpha_2$-responsiveness was greater in adults with MS when compared with controls (Figure 4). To begin to understand specific mechanisms underlying increased $\alpha_2$-mediated vasoconstriction in the current research cohort, we examined relationships between $\alpha_2$-adrenergic receptor responsiveness and MSNA.

**Relationship between $\alpha_2$-adrenergic Responsiveness and MSNA**

When the relationships between vasoconstrictor responses to clonidine and MSNA were examined both at rest and during exercise, no direct relationships were observed in either group (Figure 5). Findings from the current study suggest factors other than MSNA (i.e. altered responsiveness to local metabolites, bioavailability of sympatholytic factors, etc) play a more direct role in the observed response in human MS. Consistent with this concept, alterations in the bioavailability of NO have been shown to contribute significantly to patterns and severity of adrenergic constriction in animal models of MS (92, 211). In humans, when endothelial function was preserved in type 2 diabetics, adrenergic responsiveness during leg-extension exercise was maintained (219). Whereas endothelial function was not assessed with the current study design, research supports the presence of dysfunction in human MS (156, 210). Taken together, if the integrity of the endothelium and/or sensitivity to muscle metabolites is altered in MS, this may play a more important role in the observed increase in $\alpha_2$-mediated vasoconstriction than levels of MSNA.
In addition to altered sensitivity to muscle metabolites, it is possible adults with MS exhibit altered metabolic milieu when compared with healthy controls. Unpublished research from our laboratory suggests adults with MS exhibit higher levels of oxygen consumption at the same absolute exercise intensity when compared with healthy control subjects (see Project 1 of this dissertation). Subjects in the current study completed exercise at 15% of MVC based on research suggesting sympatholytic factors are determined by relative exercise workloads (34, 36, 57, 107). Given MVC was not significantly different between groups (Table 1), subjects completed forearm exercise at approximately the same absolute exercise intensity (~6 kg); thus, it is possible the absolute concentration of metabolites available to interfere with sympathetic vasoconstriction was higher in adults with MS when compared with healthy controls. If the absolute concentration of muscle metabolites was increased in the current cohort of MS subjects during exercise, this may have led us to underestimate group differences in $\alpha_2$-mediated vasoconstriction. However, future research is necessary to examine the relationship between absolute metabolic need and $\alpha_2$-adrenergic vasoconstriction to better understand the mechanisms behind altered vascular control in this population.

**Sympathetically-Mediated Restraint of Blood Flow During Exercise**

The ultimate effect of sympathetic nerve activity on the vasculature depends on both the strength of the activity (i.e. MSNA, neurotransmitter synthesis and release) and the vascular responsiveness to that activity (i.e. receptor number, receptor sensitivity, downstream signaling). MS adults in the current study exhibit increased MSNA when compared with healthy controls—strengthening an already large body of literature (72,
Maintained ($\alpha_1$) or increased ($\alpha_2$) adrenergic responsiveness combined with higher total MSNA may result in increased sympathetic restraint of blood flow during exercise. To explore this concept further, we inhibited endogenous $\alpha$-adrenergic vasoconstriction with phentolamine. When phentolamine was infused during exercise, adults with MS exhibited a blunted rise in conductance from steady-state exercise when compared with healthy controls (Figure 6), suggesting a relatively lower level of endogenous sympathetically-mediated vasoconstriction. We then examined the relative response to phentolamine infusion in relation to MSNA to explore the potential mechanisms behind this response; two distinct relationships were observed (Figure 7).

**Sympathetic Restraint of Exercise Blood Flow in Healthy Control Subjects**

During exercise the sympathetic nervous system plays an important role in limiting exercise blood flow, thereby preventing an increase in blood flow to active skeletal muscle from outstripping cardiac output (193). When endogenous $\alpha$-mediated vasoconstriction was inhibited in healthy adults in the present investigation, an increase in exercise FVC was observed (Figure 6). The level of increase in FVC with phentolamine infusion was linearly related to MSNA; healthy adults with the highest MSNA exhibited the greatest increase in FVC (Figure 7). This relationship is especially important during whole body exercise to maintain systemic blood pressure and oxygen delivery to vital organs (193).
Sympathetic Restraint of Exercise Blood Flow in Metabolic Syndrome

In contrast to healthy adults, there was a trend ($r=0.4$, $p=0.13$) for an inverse relationship between MSNA and sympathetic restraint in adults with MS. This was surprising. MS adults in the current study exhibited maintained $\alpha_1$- and increased $\alpha_2$-adrenergic responsiveness. Combined with increases in MSNA, we expected to observe maintained or increased vasoconstriction during exercise such that when phentolamine was infused, the rise in FVC would be similar to or greater than responses in healthy control subjects. However, physiological adaptations appear to occur in adults with MS that result in a relative reduction in $\alpha$-adrenergic tone during exercise. This may partially explain maintained whole-limb exercise blood flow in the face of higher MSNA observed in human MS.

It is unlikely blunted $\alpha$-adrenergic mediated restraint of blood flow is the result of reduced adrenergic receptor number, receptor responsiveness, or downstream signaling given adrenergic responsiveness in MS adults was maintained/enhanced at rest. Rather, we speculate altered sympathetic restraint in human MS may be due to reduced levels of endogenous norepinephrine. Chronic neural activation can result in a negative feedback relationship between pre- and post-synaptic adrenergic receptors (127, 144, 145); therefore, we propose adults with MS exhibit reduced neurotransmitter release per sympathetic burst, potentially due to pre-synaptic inhibition. Along these lines, research has shown obese women to exhibit reduced norepinephrine spillover in forearm skeletal muscle when compared with lean controls (43). In addition, animal models of diabetes and hypertension support the presence of hyperactive pre-synaptic
α₂-adrenergic receptors and/or reduced norepinephrine overflow (20, 96, 217). To further explore this possibility, we measured plasma levels of norepinephrine in the current research cohort. Norepinephrine levels were similar between groups (Control 191±22, MS 181±28 pg.mL⁻¹; p=0.39); thus, it is reasonable to propose adults with MS in the current cohort exhibited reduced plasma norepinephrine per MSNA burst when compared with healthy controls. However, it is important to note plasma concentrations are an imprecise measure of catecholamine release (71) and future research will be necessary to directly examine the potential for altered norepinephrine release in human MS.

**Integrated Neurovascular Control**

An important strength of the current study design was the direct assessment of receptor-specific responsiveness, MSNA, and sympathetic restraint. Combining multiple measures allowed us to develop an overall understanding of the mechanisms dictating neurovascular control in human MS. Given the integration of various control mechanisms, it is naïve to assume MSNA will have a direct effect on all vascular responses observed under both resting and exercise conditions. However, correlation analysis allowed us to examine interindividual variability in vascular control and the overall balance of effects in human MS.

Research in animals supports a role for α₁-adrenergic receptors in dictating whole-limb blood flow. In the present investigation, whole limb blood flow during exercise was maintained – if not increased – in adults with MS. Maintained whole-limb blood flow
during exercise was likely the result of physiological adaptations to increased MSNA in MS subjects. First, we observed an inverse relationship between α₁-mediated vasoconstriction and MSNA in MS adults; adults with high levels of MSNA exhibited reduced responsiveness to α₁-adrenergic receptor activation during exercise. Second, MS adults with high levels of MSNA exhibited reduced α-adrenergic restraint of whole-limb blood flow during exercise. Whereas specific mechanisms behind this relationship are unknown, we speculate adults with MS exhibit reduced neurotransmitter release per sympathetic burst, potentially due to increased pre-synaptic α₂-adrenergic inhibition. Taken together, whole limb blood flow was increased during exercise in MS subjects as a result of individual-specific changes in neurovascular control.

In contrast with α₁-adrenoceptors, post-synaptic α₂-adrenergic receptors are thought to determine blood flow distribution and oxygen delivery within the skeletal muscle. MS adults in the current study exhibited increased α₂-adrenergic responsiveness that was not directly related to MSNA. Despite maintained whole-limb blood flow, MS adults potentially exhibit altered flow distribution due to non-adrenergic factors. Such findings emphasize the complexity of processes controlling blood flow, oxygen delivery and exercise tolerance in health and disease.

**Experimental Considerations**

We assessed group differences in α-adrenergic responses by local infusion of receptor-specific pharmacologic agonists. Phenylephrine is a well-accepted, specific α₁-adrenergic agonist; however it may cause transient β₂-adrenergic vasodilation (222).
Given MS subjects may exhibit blunted β_2-mediated vasodilation (150), the proposed study was conducted with continuous β-adrenergic blockade using doses previously shown to sufficiently block β-receptor activation (66, 122). Clonidine, on the other hand, may act post-synaptically on the vascular smooth muscle, in addition to pre-synaptically on sympathetic nerve terminals (244). Activation pre-synaptically would inhibit norepinephrine release, potentially limiting conclusions specific to α_2-adrenergic vasoconstriction. However, research suggests the primary effects of clonidine are likely at post-junctional α_2-receptors (18) and previous research using tyramine and clonidine infusion supports the primary action of clonidine post-junctionally (55, 56, 60). Taken together, the specific pharmacological agents chosen for the current study were appropriate. However, it is important to note adrenergic agonists were delivered intra-arterially (luminal) and may not reflect responses of adrenergic receptors normally stimulated by norepinephrine released from nerve endings (abruminal) (120). Therefore, we assessed endogenous α-adrenergic mediated vasoconstriction with infusion of non-selective α-adrenergic antagonist, phentolamine.

We were surprised to observe a blunted relative increase in FVC with phentolamine infusion in adults with MS during dynamic steady-state exercise. As mentioned above, this may be the result of reduced α_1-adrenergic responsiveness in relation to increased MSNA and/or reduced endogenous norepinephrine. It is also possible non-adrenergic sympathetic restraint of blood flow may exist that was not examined with the current design; specifically, neuropeptide Y and ATP (non-adrenergic neurotransmitters released from sympathetic nerve terminals) have been shown to cause vasoconstriction.
in the exercising muscle (16, 17). Non-adrenergic mediated vasoconstriction has been shown to be increased with aging (49), however future research will be necessary to examine its role during exercise in human MS.

Linear regression analysis of the relationship between forearm adrenergic responsiveness and MSNA was conducted using measures of MSNA (microneurography of the peroneal nerve) collected at rest on the second study day. We feel our study design was appropriate for the following reasons. First, MSNA has been shown to be repeatable between days and across years (33, 235). Second, MSNA remains at resting levels during dynamic forearm exercise at 15% MVC (231) (Appendix C7). Last, upper extremity MSNA and lower extremity MSNA appear to be uniform under resting conditions in both healthy controls and obese adults (2). Taken together, the current study design is not limiting; however, it is important to note research exploring neurovascular changes with aging have shown reduced sympathetic vascular tone in the resting forearms whereas α-adrenergic vasoconstriction may be increased in the leg (55, 56, 60). Therefore, conclusions gathered from the current study are specific to the vascular bed and exercise intensity examined.

Both men and women were included in the current study. In recent years, research has emerged in the area of sex-specificity of neurovascular control. Previous research from our lab indicates sex differences do not exist in forearm blood flow and/or adrenergic responsiveness during low intensity (15% MVC) exercise in healthy adults (61, 82, 153). However, menstrual-phase related differences may exist in neurovascular control. To
control for this potential difference, all women participated during the early follicular (days 1-6) phase of the female menstrual cycle; therefore, we do not feel including both sexes is a limitation. However, future research will be necessary to explore the potential for sex-specific differences in neurovascular control in human MS.

MS is a clustering of cardiovascular disease risk factors that increases a person’s risk of developing cardiovascular disease above and beyond that of each individual factor. When results of primary outcome variables were adjusted for metabolic syndrome criteria post hoc (waist circumference, glucose, HDL cholesterol, triglycerides), the effect of group on adrenergic responsiveness and sympathetic restraint was maintained; thus, it appears the clustering of risk factors in MS may play a more significant role in neurovascular control than each individual component. When individual factors were examined previously by other investigators, α-adrenergic mediated vasoconstriction was maintained [waist circumference, hyperglycemia; (2, 230)], reduced [hyperinsulinemia; (149)], and/or increased [hypertension, dyslipidemia; (63, 234)]. Future studies will be necessary to further examine the physiological effects of MS as both a whole and its parts.

**Conclusion**

Whereas previous work in animals has identified multiple signaling pathways that may be altered in metabolic syndrome, this is the first study to translate such findings to human MS. By combining multiple measures (MSNA, α-adrenergic receptor responsiveness, and α-adrenergic restraint) both at rest and during exercise, we were
able to develop an overall understanding of receptor-specific alterations in neurovascular control in human MS. Novel findings from the current study highlight the importance of an integrative approach to research examining vascular control in both health and disease.

Adults with MS exhibited increased α2-mediated vasoconstriction during exercise when compared with healthy controls; however no direct relationship was observed between α2-adrenergic responsiveness and MSNA. These findings suggest other factors (such as altered responsiveness to local metabolites or bioavailability of sympatholytic factors) possibly play a more direct role in the observed response in human MS. In contrast, group differences in α1-adrenergic responsiveness were not detected, however a linear relationship was observed between MSNA and α1-mediated vasoconstriction in MS adults. Taken together with blunted sympathetic restraint, blunted α1-mediated vasoconstriction may be an important adaptation resulting in maintained whole-limb blood flows during exercise. The impact of these findings on flow distribution, oxygen consumption, and exercise tolerance in this population is currently unknown. Future work will be necessary to elucidate the mechanisms behind receptor-specific neurovascular control and the progression of such adaptations with increasing cardiovascular disease risk.
ACKNOWLEDGEMENTS

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### Table 2.1: Subject Demographics

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<tr>
<td>Age (years)</td>
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<td>MSNA Burst Incidence (burst/100 HR)</td>
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<td>Desaturation Event Index (event/hr)</td>
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Data are presented as Mean ± SE. Control n=16, MS n=14 unless otherwise noted (Desaturation Event Index: Control n=15, MS n=13; MSNA measures: Control n=12, MS n=10), *p<0.05 vs Control.

BMI: Body Mass Index, HDL: High-density Lipoprotein, MSNA: muscle sympathetic nerve activity, HR: heart rate, MVC: Maximal Voluntary Contraction
Table 2.2: Forearm blood flow during forearm exercise prior to β-adrenergic blockade

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<td>Heart Rate (beat/min)</td>
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<td></td>
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<tr>
<td></td>
<td>Exercise (min 7)</td>
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<td>25±2 a</td>
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</tbody>
</table>

Table 2.2: Forearm blood flow during forearm exercise prior to β-adrenergic blockade

Data are presented as Mean ± SE. Control n=16, MS n=14

Main effect of group (* p<0.05 vs Control); Main effect of exercise (a p<0.05 vs Rest);

Interaction between group and exercise († p<0.05 vs Control at a given workload)
### Table 2.3: Responses to exercise and phenylephrine infusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (beat/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>57±2</td>
<td>65±3*</td>
</tr>
<tr>
<td>Exercise</td>
<td>61±2</td>
<td>69±3*</td>
</tr>
<tr>
<td>Exercise + PE</td>
<td>60±2</td>
<td>69±3*</td>
</tr>
<tr>
<td>Mean Blood Pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>91±2</td>
<td>109±4*</td>
</tr>
<tr>
<td>Exercise</td>
<td>92±2</td>
<td>111±4*</td>
</tr>
<tr>
<td>Exercise + PE</td>
<td>96±2</td>
<td>118±5*</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.43±0.02</td>
<td>0.44±0.02*</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.43±0.02</td>
<td>0.46±0.02*</td>
</tr>
<tr>
<td>Exercise + PE</td>
<td>0.44±0.02</td>
<td>0.46±0.02*</td>
</tr>
<tr>
<td>Blood Flow (mL/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>50±10</td>
<td>103±16</td>
</tr>
<tr>
<td>Exercise</td>
<td>207±19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>392±46&lt;sup&gt;†a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Exercise + PE</td>
<td>188±18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>370±46&lt;sup&gt;†a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Change with PE (%)</td>
<td>-8±4</td>
<td>-6±4</td>
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<tr>
<td>Vascular Conductance (mL/min*100mmHg)</td>
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<tr>
<td>Rest</td>
<td>53±10</td>
<td>94±13</td>
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<tr>
<td>Exercise</td>
<td>226±21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>351±36&lt;sup&gt;†a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Exercise + PE</td>
<td>197±18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>307±33&lt;sup&gt;†a&lt;/sup&gt;</td>
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<tr>
<td>Change with PE (%)</td>
<td>-11±4</td>
<td>-12±3</td>
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</tbody>
</table>

Data are presented as Mean ± SE. Control n=16, MS n=13 (1 MS subject did not complete due to discomfort during initial PE infusion). PE = phenylephrine

Main effect of group (* p<0.05 vs Control); Main effect of exercise (<sup>a</sup> p<0.05 vs Rest, <sup>b</sup> p<0.05 vs Exercise); Interaction between group and exercise (<sup>†</sup> p<0.05 vs Control at a given workload)
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate (beat/min)</strong></td>
<td>55±2</td>
<td>66±3*</td>
</tr>
<tr>
<td>Rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>61±2</td>
<td>69±3*</td>
</tr>
<tr>
<td>Exercise + CL</td>
<td>60±2</td>
<td>70±3*</td>
</tr>
<tr>
<td><strong>Mean Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>92±2</td>
<td>108±4*</td>
</tr>
<tr>
<td>Exercise</td>
<td>91±2</td>
<td>109±4*</td>
</tr>
<tr>
<td>Exercise + CL</td>
<td>95±2</td>
<td>116±5*</td>
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<tr>
<td><strong>Diameter (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.43±0.02</td>
<td>0.43±0.02</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.44±0.02</td>
<td>0.45±0.02</td>
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<tr>
<td>Exercise + CL</td>
<td>0.44±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td><strong>Blood Flow (mL/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>47±12</td>
<td>102±11</td>
</tr>
<tr>
<td>Exercise</td>
<td>218±19a</td>
<td>391±49†a</td>
</tr>
<tr>
<td>Exercise + CL</td>
<td>184±16a</td>
<td>292±40†a</td>
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<td>Change with CL (%)</td>
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<td><strong>Vascular Conductance (mL/min*100mmHg)</strong></td>
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<tr>
<td>Rest</td>
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<td>Exercise</td>
<td>240±22a</td>
<td>337±35†a</td>
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<td>Exercise + CL</td>
<td>196±18a</td>
<td>239±26a, b</td>
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<td>Change with CL (%)</td>
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<td>-31±2*</td>
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Table 2.4: Responses to exercise and clonidine infusion

Data are presented as Mean ± SE. Control n=16, MS n=14. CL= clonidine

Main effect of group (* p<0.05 vs Control); Main effect of exercise (a p<0.05 vs Rest, b p<0.05 vs Exercise); Interaction between group and exercise († p<0.05 vs Control at a given workload)
Table 2.5: Responses to exercise and phentolamine infusion

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<th>Metabolic Syndrome</th>
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<td><strong>Heart Rate</strong></td>
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<td></td>
</tr>
<tr>
<td>(beat/min)</td>
<td>Rest</td>
<td>56±2</td>
</tr>
<tr>
<td></td>
<td>Exercise</td>
<td>59±2</td>
</tr>
<tr>
<td></td>
<td>Exercise + PH</td>
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<tr>
<td><strong>Mean Blood Pressure</strong></td>
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<td></td>
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<tr>
<td>(mmHg)</td>
<td>Rest</td>
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<td></td>
<td>Exercise</td>
<td>89±2</td>
</tr>
<tr>
<td></td>
<td>Exercise + PH</td>
<td>90±3</td>
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<tr>
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<tr>
<td>(cm)</td>
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<td></td>
<td>Exercise</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td></td>
<td>Exercise + PH</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td><strong>Blood Flow</strong></td>
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<td></td>
</tr>
<tr>
<td>(mL/min)</td>
<td>Rest</td>
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<tr>
<td></td>
<td>Exercise</td>
<td>201±19**</td>
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<td></td>
<td>Exercise + PH</td>
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<td>Change with PH (Δ)</td>
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<td></td>
<td>Change with PH (%)</td>
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<tr>
<td><strong>Vascular Conductance</strong></td>
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<tr>
<td>(mL/min*100mmHg)</td>
<td>Rest</td>
<td>70±19</td>
</tr>
<tr>
<td></td>
<td>Exercise</td>
<td>227±23**</td>
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<td></td>
<td>Exercise + PH</td>
<td>417±48**</td>
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<td>Change with PH (Δ)</td>
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<td>Change with PH (%)</td>
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</tbody>
</table>

Data are presented as Mean ± SE. Control n=16, MS n=11 (3 MS subjects did not receive PH due to drug shortage). PH= phentolamine

Main effect of group (* p<0.05 vs Control); Main effect of exercise (** p<0.05 vs Rest, a p<0.05 vs Exercise); Interaction between group and exercise (** p<0.05 vs Control at a given workload)
Figure 2.1: (A) Lean forearm vascular conductance (Lean FVC) and (B) the rise in Lean FVC from rest during 4 minutes of steady-state exercise (∆Lean FVC) in healthy controls and adults with metabolic syndrome.  

Control n=16, MS n=14.  FVC normalized to lean muscle mass (Lean FVC) increased from rest to steady-state exercise [Main effect of workload (a p<0.05 vs Rest)].  

Measures of lean FVC at steady-state exercise (A) as well as the rise in lean FVC from rest (B) were both greater in MS adults when compared with healthy controls [Main effect of group (* p<0.05 vs Control)].
Figure 2.2: Relative reduction in vascular conductance with infusion of $\alpha_1$-adrenergic receptor agonist, phenylephrine.

Control n=16, MS n=13. REST: Phenylephrine infusion resulted in a significant reduction in FVC at rest and relative responses (%FVC) were not different between groups. EXERCISE: During exercise, phenylephrine-mediated vasoconstriction (%FVC) was blunted from resting levels [Main effect of exercise ($^a$p<0.05 vs Rest)] and the level of vasoconstriction was similar between groups.
Figure 2.3: The relationship between MSNA Burst Incidence and vascular responses to Phenylephrine infusion in healthy controls and adults with metabolic syndrome.

Control n=12, MS n=9. REST: An inverse linear relationship between resting $\alpha_1$-adrenergic responsiveness and MSNA was observed in young healthy adults; adults with higher MSNA exhibited blunted $\alpha_1$-vasoconstrictor responses. A relationship was not detected in MS subjects at rest. EXERCISE: There was no observed relationship
between MSNA and $\alpha_1$-adrenergic responsiveness during exercise in healthy adults, however MS adults exhibited reduced vasoconstrictor responses with higher levels of MSNA.
Figure 2.4: Relative reduction in vascular conductance with infusion of $\alpha_2$-adrenergic receptor agonist, clonidine.

Control n=16, MS n=14. REST: Clonidine infusion resulted in a significant reduction in FVC at rest, with relative responses (%FVC) greater in adults with MS when compared with healthy controls [Main effect of group (*p<0.05)]. EXERCISE: During exercise, clonidine-mediated vasoconstriction (%FVC) was blunted from resting levels [Main effect of exercise ($^a$p<0.05 vs Rest)] and the level of vasoconstriction was greater in adults with MS when compared with healthy controls [Main effect of group (*p<0.05)].
Figure 2.5: The relationship between MSNA Burst Incidence and vascular responses to Clonidine infusion in healthy controls and adults with metabolic syndrome.

Control n=12, MS n=10. REST and EXERCISE: No relationship was observed between MSNA and clonidine-mediated vasoconstriction at rest or during exercise in either group.
Figure 2.6: (A) Lean forearm vascular conductance (Lean FVC), and (B) the relative rise in Lean FVC from steady-state exercise with Phentolamine infusion (% Lean FVC) in healthy controls and adults with metabolic syndrome.

Control n=16, MS n=13. FVC normalized to lean muscle mass (Lean FVC) was greater in MS adults when compared with healthy control subjects [Main effect of group (*p<0.05 vs Control)]. Lean FVC increased from rest to steady-state exercise [Main effect of workload (*a p<0.05 vs Rest)]. Phentolamine infusion during exercise resulted in a significant increase in FVC [Main effect of workload (*b p<0.05 vs Steady-state exercise)]. The relative rise in lean FVC (% Lean FVC) with phentolamine infusion [(FVC_{phentolamine} - FVC_{steady-state exercise}) + FVC_{steady-state exercise} x 100%] was lower in MS adults when compared with healthy control subjects.
Figure 2.7: The relationship between MSNA Burst Incidence and relative vascular responses to Phentolamine infusion during exercise (% Lean FVC) in healthy controls and adults with metabolic syndrome.

Control n=12, MS n=8. In healthy controls, MSNA was positively related to sympathetic restraint—subjects with high MSNA exhibited a greater relative increase in FVC with infusion of phentolamine ($r=0.5$, $p=0.05$). However, there was a trend for the inverse relationship in adults with MS; those with higher MSNA exhibited blunted sympathetic restraint ($r=-0.4$, $p=0.13$).
CONCLUSION

Metabolic Syndrome

Metabolic syndrome (MS) is present in nearly one third of the United States population. Adults with MS are obese, hypertensive, hyperglycemic, dyslipidemic, and at high risk of developing cardiovascular disease and diabetes. Physical activity is an effective non-pharmacological way to combat the syndrome. Research in animal models of MS suggests blood flow responses to exercise are impaired due to altered vascular control mechanisms. Whereas previous work in animals has identified multiple signaling pathways that may be altered in MS, this is the first series of studies to attempt to translate such findings to human MS.

Specific Aims and Hypotheses

The overall goal of this dissertation was to gain insight into exercise blood flow control in human MS. The specific aims were to determine: 1) whether adult humans with MS exhibit blunted exercise-induced skeletal muscle blood flow, 2) whether blood flow responses in MS are attenuated with additional physiological stress, 3) whether adults with MS exhibit increased α-adrenergic vasoconstriction during exercise, and 4) whether α-adrenergic vasoconstriction during exercise is related to muscle sympathetic nerve activity (MSNA).

We hypothesized skeletal muscle blood flow during exercise would be lower in adults with MS when compared with healthy controls. Additionally, we hypothesized the rise in exercise blood flow due to hypoxia would be blunted in adults with MS. The exact
mechanisms behind such differences are unknown. Based on experimental evidence, we hypothesized adults with MS would exhibit increased \(\alpha\)-adrenergic vasoconstriction during exercise. Lastly, we hypothesized this level of vasoconstriction during exercise would be positively related to MSNA.

**Blood Flow Responses to Exercise and Hypoxia in Human Metabolic Syndrome**

We have shown for the first time that blood flow responses to forearm exercise are increased in human MS. Whereas this may be due to increased adipose tissue blood flow and/or impaired flow distribution, group differences are most likely the result of increased metabolic need at the same absolute workloads in MS. The mechanisms behind increased oxygen consumption at the same absolute workload in human MS are largely unexplored. Future work will be necessary to elucidate the physiological need for such an adaptation in human MS, the mechanisms behind such changes, and the effect such changes have on vascular responses under conditions of greater metabolic need (i.e. higher exercise intensities and/or enhanced physiological stress).

To explore this further, the present investigation examined vascular responses during exercise under hypoxic conditions in human MS. Hypoxia provides an enhanced metabolic signal relative to exercise alone that challenges oxygen delivery and alters sympathetic tone. Contrary to our hypothesis, we observed maintained hypoxic compensatory vasodilation during exercise in MS adults when compared with healthy controls. Despite tightly controlled experimental conditions, vascular responses to hypoxia were highly variable both within and between groups. Such heterogeneous responses were likely due
to individual-specific relationships between sympathetically-mediated vasoconstriction and local vasodilation. Findings highlight the importance of integrative vascular control in both health and disease. Future work will be necessary to elucidate specific physiological mechanisms responsible for heterogeneity in vascular responses to hypoxia.

**Sympathetic Control of Blood Flow during Exercise in Human Metabolic Syndrome**

By combining multiple measures (receptor-specific responsiveness, MSNA, and α-adrenergic restraint) both at rest and during exercise, we were able to develop an overall understanding of receptor-specific neurovascular control in human MS. Adults with MS exhibited increased α2-mediated vasoconstriction during exercise when compared with healthy controls; however no direct relationship was observed between α2-adrenergic responsiveness and MSNA. These findings suggest other factors (such as altered responsiveness to local metabolites or bioavailability of sympatholytic factors) possibly play a more direct role in the observed response in human MS. In contrast, group differences in α1-adrenergic responsiveness were not detected, however a linear relationship was observed between MSNA and α1-mediated vasoconstriction in MS adults that, taken together with blunted sympathetic restraint, may be an important adaptation resulting in maintained whole-limb blood flows during exercise. Future work will be necessary to elucidate the mechanisms behind receptor-specific neurovascular control and the progression of such adaptations with increasing cardiovascular disease risk.
Conclusion

This is the first series of studies that attempted to translate previous work in animal models of MS to humans. The projects included in this dissertation provide a better understanding of the pathophysiology of MS and highlight the importance of integrative vascular control in both health and disease. In addition, results provide translationally relevant outcomes that will be useful in designing and interpreting future studies in human MS.
APPENDIX A: STUDY TIMELINES
TIMELINE: Blood Flow Responses to Exercise and Hypoxia in Human Metabolic Syndrome

After insertion of the venous catheter, four (4) individual trials (8 and 12 kg exercise under normoxic and hypoxia) were conducted in random order. Steady-state ventilation at the desired $S_pO_2$ was maintained at rest for an average of 4 minutes. Subjects then completed 3.5 minutes of dynamic exercise (20 contractions/min). Blood velocity, blood pressure, heart rate, and oxygen saturation measures (▱) were reported from the last 30 seconds of both rest and exercise, followed by an image of the brachial artery (↓, analyzed for vessel radius). A minimum of 10 minutes of normoxic rest separated each trial.
TIMELINE: Sympathetic Control of Blood Flow During Exercise in Human Metabolic Syndrome

After arterial catheter insertion, eight (8) individual trials were conducted. Arterial diameter (↓) and steady-state measures of blood velocity, blood pressure, and heart rate (█) were taken during the last 30 seconds of rest/exercise/drug infusion. Trial 1 was an exercise control trial and during Trial 2 propranolol (PR) was administered for β-adrenergic blockade (a maintenance dose was given for the remainder of the trials). Trials 3-6 were randomized and counterbalanced (PE = phenylephrine, CL = clonidine). Trial 7 assessed sympathetic restraint (PH = phentolamine) and Trial 8 verified α-adrenergic blockade. Trials were separated by a minimum of 10 minutes of quiet rest.
AIM 1: Exercise-Induced Skeletal Muscle Blood Flow in Human Metabolic Syndrome

Heart rate (electrocardiogram), blood pressure (finger photoplethysmography), blood velocity (Doppler ultrasound), and oxygen saturation (not shown) were collected continuously throughout rest and exercise. The figure is an example of 10 seconds of data during normoxic forearm exercise in an adult with MS.
**AIM 2: Hypoxia-Mediated Vasodilation in Human Metabolic Syndrome.**

Heart rate (electrocardiogram), blood pressure (finger photoplethysmography), blood velocity (Doppler ultrasound), and oxygen saturation (pulse oximetry) were collected continuously throughout rest and exercise. The above figure is an example of 20 minutes of condensed data from an adult with MS. Data are presented as follows: rest before hypoxia, rest during the transition to hypoxia, rest during hypoxia, 3.5 minutes of dynamic exercise at 8 kg under hypoxic conditions.
**AIM 3: Sympathetic Control of Blood Flow During Exercise in Human Metabolic Syndrome.**

Heart rate (electrocardiogram), blood pressure (indwelling catheter), and blood velocity (Doppler ultrasound) were collected continuously throughout rest and exercise. The above figure is an example of 10 minutes of condensed data collected from a healthy control. Data are presented as follows: quiet rest, exercise at 15% MVC, exercise at 15% MVC with intra-arterial infusion of phenylephrine (an $\alpha_1$-adrenergic agonist).
AIM 4: The Relationship Between α-adrenergic Responsiveness and MSNA in Human Metabolic Syndrome.

Heart rate (electrocardiogram), blood pressure (finger photoplethysmography), blood velocity (Doppler ultrasound), ventilation (strain gauge), and muscle sympathetic nerve activity (microneurography, integrated and raw neurogram) were collected continuously throughout rest and dynamic exercise. The above figure is an example of 20 seconds of data collected in a subject with MS during dynamic forearm exercise at 15% MVC.
APPENDIX B: METABOLIC SYNDROME CRITERIA
## Project 1: Metabolic Syndrome Criteria

<table>
<thead>
<tr>
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<td>X</td>
<td>X</td>
<td>6</td>
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</tbody>
</table>

| # of Criteria      | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Adults were characterized as having MS if, after in-person assessment, subjects met at least three of the following National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria as modified by the American Diabetes Association: central obesity (waist circumference >102 cm males, >88 cm females), pre-hypertension (resting blood pressure ≥130/≥85 mmHg), hypertriglyceridemia (triglycerides ≥150 mg/dL), hyperglycemia (fasting glucose ≥100 mg/dL) and/or dyslipidemia (HDL <40 mg/dL males, <50 mg/dL females) (4, 98).
### Project 2: Metabolic Syndrome Criteria

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<td>Men &gt;102 cm</td>
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<td>Women &gt;88 cm</td>
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<td>X</td>
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<td><strong>2. HDL</strong></td>
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<tr>
<td>Men &lt;40 mg/dL</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Women &lt;50 mg/dL</td>
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<td>12</td>
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<tr>
<td>≥130/≥85 mmHg</td>
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<td><strong>4. Glucose</strong></td>
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<td>≥100 mg/dL</td>
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<td><strong>5. Triglycerides</strong></td>
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<td>≥150 mg/dL</td>
<td>X</td>
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</table>

Adults were characterized as having MS if, after in-person assessment, subjects met at least three of the following National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria as modified by the American Diabetes Association: central obesity (waist circumference >102 cm males, >88 cm females), pre-hypertension (resting blood pressure ≥130/≥85 mmHg), hypertriglyceridemia (triglycerides ≥150 mg/dL), hyperglycemia (fasting glucose ≥100 mg/dL) and/or dyslipidemia (HDL <40 mg/dL males, <50 mg/dL females) (4, 98).
Appendix C1: The relationship between forearm volume and lean muscle mass

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=22)</th>
<th>Obese (n=50)</th>
</tr>
</thead>
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<tr>
<td>Sex (M/F)</td>
<td>14/8</td>
<td>25/25</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>29±2</td>
<td>43±2</td>
</tr>
<tr>
<td>Total Body Fat (%)</td>
<td>22±2</td>
<td>43±1</td>
</tr>
<tr>
<td>Total Forearm Volume (mL)</td>
<td>941±41</td>
<td>1333±44</td>
</tr>
<tr>
<td>Total Forearm Tissue (g)</td>
<td>1016±49</td>
<td>1333±51</td>
</tr>
<tr>
<td>Forearm Fat (%)</td>
<td>9±2</td>
<td>29±2</td>
</tr>
<tr>
<td>Forearm Fat (g)</td>
<td>89±14</td>
<td>386±22</td>
</tr>
<tr>
<td>Forearm Lean Mass (g)</td>
<td>927±52</td>
<td>956±45</td>
</tr>
<tr>
<td>Forearm Lean Mass (g) =</td>
<td>[1.1 x (FAV, mL)] – 115</td>
<td>[0.7 x (FAV, mL)] + 75</td>
</tr>
</tbody>
</table>

\[
y = 1.1088x - 115.49 \\
R^2 = 0.7863
\]

\[
y = 0.6604x + 75.494 \\
R^2 = 0.4331
\]
Appendix C1: Previous research has shown forearm volume as measured with water displacement (FAV, mL) and lean forearm muscle mass (g) to be closely related in young, lean adults (159). Given greater adiposity in adults with metabolic syndrome (MS), we examined the relationships between FAV and lean mass from data collected from lean adults and obese adults. We compared forearm volume as measured with water displacement (mL) and lean forearm mass as measured by whole-body dual-energy x-ray absorptometry (DEXA; GE Lunar Prodigy; Milwaukee, WI). Linear regression analysis provided two separate regression equations that can be used to estimate lean mass from non-invasive FAV measures in lean and obese adults.
Appendix C2: Venous blood gasses and time-to-analysis

For Project 1, venous blood samples (n=4) were drawn in duplicate, placed on ice, and analyzed within 2 hours (120 minutes) of collection for measurements of pH and the partial pressures of oxygen ($P_{vO2}$) and carbon dioxide ($P_{vCO2}$) using a blood-gas analyzer calibrated with tonometered blood (ABL500; Radiometer, Copenhagen, Denmark). Pilot work showed no detectable difference between average measurements analyzed 30 or 150 minutes after collection (p>0.05).
Appendix C3: The relationship between end-tidal carbon dioxide levels and forearm hypoxic-mediated vasodilation

A potential limitation of Project 1 was the slightly lower end-tidal carbon dioxide (CO$_2$) levels that occurred during hypoxic trials. Given previous research showing the reduction in oxygen to be the primary mechanism for vasodilation during acute hypoxia, regardless of CO$_2$ levels (112), this was a minor limitation of the current study. To support this conclusion, we examined changes in end-tidal CO$_2$ levels and the potential relationship with hypoxia-mediated vasodilation. A significant relationship between end-tidal CO$_2$ and compensatory hypoxic vasodilation was not detected when data were analyzed together ($r=0.1$, $p=0.36$) or by workload (see above Figure). Taken together, we do not feel changes in CO$_2$ altered our conclusions regarding vascular responses to hypoxia.
Appendix C4: Resting Blood Flow Responses to Hypoxia in Human Metabolic Syndrome.

Control n=13, MS n=8. Although not a primary focus of the current study, vascular responses to hypoxia at rest were greater in adults with MS (Main effect of group, p<0.05). Specifically, those subjects with MS exhibited greater hypoxic-mediated vasodilation when compared with healthy control subjects.
Appendix C5: Repeatability of Vascular Conductance

Control $n=15$, MS $n=10$. Data are presented as Mean ± SE. Forearm exercise for both Project 1 and Project 2 was completed at mild-to-moderate exercise intensities (~15-30% of maximal intensity, ~6-12 kg). Our lab has shown vascular responses to exercise at 15% MVC to be repeatable across exercise trials spanning ~120 minutes (Main effect of trial, $p=0.955$).

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>% CV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular Conductance</td>
<td>Control</td>
<td>226±21</td>
<td>240±22</td>
<td>227±23</td>
<td>11±1</td>
</tr>
<tr>
<td>(mL/min*100mmHg)</td>
<td>Metabolic Syndrome</td>
<td>346±39</td>
<td>348±36</td>
<td>357±38</td>
<td>11±2</td>
</tr>
</tbody>
</table>
Appendix C6: Confirmation of pharmacological inhibition of α-adrenergic vasoconstriction by phentolamine (PH) using phenylephrine (PE).

Control n=13, MS n=10. In Project 2, phenylephrine (PE) infusion during rest resulted in a relative reduction in vascular conductance (%Change FVC). Following phentolamine (PH) infusion during exercise (Trial 7), a control trial with phenylephrine infusion was conducted (Rest+PH+PE, Trial 8) to confirm pharmacological inhibition of adrenergic vasoconstriction. There was a main effect of phentolamine infusion of vascular responses to phenylephrine [Main effect of condition, p=0.04].
Appendix C7: Change in MSNA with dynamic forearm exercise

In Project 2, a mild exercise workload (15% of maximum, ~6 kg) was used to minimize systemic effects of exercise (i.e. increases in MSNA). In addition, resting measures of MSNA were used for all comparisons; this decision is supported by previous research in healthy adults (231) and the current study (Control n=12, MS n=9) showing MSNA during dynamic forearm exercise to remain at resting levels. We observed a main effect of group (p<0.01), however there was no effect of exercise (p=0.45) nor an interaction between group and exercise (p=0.44).
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate (beat/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>56±2</td>
<td>64±3*</td>
</tr>
<tr>
<td>Rest + PE</td>
<td>57±2</td>
<td>64±3*</td>
</tr>
<tr>
<td><strong>Mean Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>90±2</td>
<td>107±3*</td>
</tr>
<tr>
<td>Rest + PE</td>
<td>92±2</td>
<td>110±3*</td>
</tr>
<tr>
<td><strong>Diameter (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.43±0.02</td>
<td>0.44±0.02*</td>
</tr>
<tr>
<td>Rest + PE</td>
<td>0.43±0.02</td>
<td>0.44±0.02*</td>
</tr>
<tr>
<td><strong>Blood Flow (mL/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>58±12</td>
<td>116±21*</td>
</tr>
<tr>
<td>Rest + PE</td>
<td>48±10</td>
<td>73±9*</td>
</tr>
<tr>
<td>Change with PE (%)</td>
<td>-23±4</td>
<td>-31±4</td>
</tr>
<tr>
<td><strong>Vascular Conductance (mL/min*100mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>64±13</td>
<td>107±18*</td>
</tr>
<tr>
<td>Rest + PE</td>
<td>51±11</td>
<td>66±8*</td>
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<tr>
<td>Change with PE (%)</td>
<td>-25±4</td>
<td>-33±4</td>
</tr>
</tbody>
</table>

**Appendix C8: Responses to phenylephrine infusion at rest**

Data are presented as Mean ± SE. Control n=16, MS n=13 (1 MS subject did not complete due to discomfort during initial PE infusion). PE = phenylephrine

Main effect of group (* p<0.05 vs Control)
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate (beat/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>58±2</td>
<td>64±3*</td>
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<tr>
<td>Rest + CL</td>
<td>57±2</td>
<td>65±4*</td>
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<tr>
<td><strong>Mean Blood Pressure (mmHg)</strong></td>
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<tr>
<td>Rest</td>
<td>91±2</td>
<td>105±4*</td>
</tr>
<tr>
<td>Rest + CL</td>
<td>92±2</td>
<td>109±4*</td>
</tr>
<tr>
<td><strong>Diameter (cm)</strong></td>
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<tr>
<td>Rest</td>
<td>0.42±0.02</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>Rest + CL</td>
<td>0.42±0.02</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td><strong>Blood Flow (mL/min)</strong></td>
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<tr>
<td>Rest</td>
<td>76±17</td>
<td>118±16*</td>
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<tr>
<td>Rest + CL</td>
<td>37±7</td>
<td>42±6*</td>
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<tr>
<td>Change with CL (%)</td>
<td>-42±5</td>
<td>-61±6*</td>
</tr>
<tr>
<td><strong>Vascular Conductance (mL/min*100mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>82±18</td>
<td>110±12*</td>
</tr>
<tr>
<td>Rest + CL</td>
<td>40±8a</td>
<td>40±6*</td>
</tr>
<tr>
<td>Change with CL (%)</td>
<td>-43±4</td>
<td>-62±5*</td>
</tr>
</tbody>
</table>

**Appendix C9: Responses to clonidine infusion at rest**

Data are presented as Mean ± SE. Control n=16, MS n=14. CL=clonidine.

Main effect of group (* p<0.05 vs Control), Main effect of condition (a p<0.05 vs Rest).
Appendix C10: Relationship between plasma measures and muscle sympathetic nerve activity (MSNA).

Control n=12, MS n=10. (A) Norepinephrine. A linear relationship was observed between burst incidence (bursts/100 heart beats) and plasma levels of norepinephrine in both healthy controls ($r=0.5$, $p=0.06$) and adults with metabolic syndrome ($r=0.5$, $p=0.07$). (B) Epinephrine. A linear relationship was not detected between plasma levels of epinephrine and burst incidence in either group (Healthy controls $r=-0.2$, $p=0.27$; MS $r=0.4$, $p=0.14$). (C) Insulin. A linear relationship was observed between fasting levels of plasma insulin and burst incidence in both healthy controls ($r=0.7$, $p<0.01$) and adults with metabolic syndrome ($r=0.5$, $p=0.08$). (D) Leptin. There was a trend for a linear relationship between fasting levels of plasma leptin and burst
incidence when both groups were combined, however a relationship was not detected in
groups when assessed independently (Healthy controls $r=0.1$, $p=0.38$; MS $r=0.1$, $p=0.42$).
APPENDIX D: HEALTH SCREENING QUESTIONNAIRE
AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health needs by marking/circling all **true** statements.

**History**

You have had:
-- a heart attack
-- heart surgery
-- cardiac catheterization
-- coronary angioplasty (PTCA)
-- pacemaker, implantable cardiac defibrillator, rhythm disturbance
-- heart valve disease
-- heart failure
-- heart transplantation
-- congenital heart disease

*If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise. You may need to use a facility with a medically qualified staff.*

**Symptoms**

-- You experience chest discomfort with exertion.
-- You experience unreasonable breathlessness.
-- You experience dizziness, fainting, blackouts.
-- You take heart medications

**Other health issues:**

-- You have musculoskeletal problems.
-- You have concerns about the safety of exercise.
-- You take prescription medication(s).
-- You are pregnant.

*If you marked two or more of the statements in this section, you should consult your healthcare provider before engaging in exercise. You might benefit by using a facility with a professionally qualified exercise staff to guide your exercise program.*

**Cardiovascular risk factors**

-- You are a man older than 45 years.
-- You are a woman older than 55 years or you have had a hysterectomy or are postmenopausal.
-- You smoke.
-- Your blood pressure is greater than 140/90.
-- You don’t know your blood pressure.
-- You take blood pressure medication.
-- Your blood cholesterol level is >240 mg/dL.
-- You don’t know your cholesterol level.
-- You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
-- You are diabetic or take medicine to control your blood sugar.
-- You are physically inactive (i.e., you get less than 30 minutes of physical activity on at least 3 days per week).
-- You are more than 20 pounds overweight.

**None of the above is true.**
- Are you currently enrolled in any other studies? __________
- Completion date of last study ______________
- Dates of future studies ________________
- Do you donate blood      No  Yes
- Date of last blood donation: __________
- Any future blood donation appointments scheduled: ________________

Female Subjects Only
Are you pregnant?      No  Yes

We will provide a urine pregnancy test to you on your first visit to the laboratory.

Oral contraceptives      No  Yes
Type: ____________________
Date you start a new pack ________________

Date of last menstrual cycle ________________

General Medical Health to Match Inclusion/Exclusion Criteria

Do you experience any of the following, or did a doctor indicate you have any of the following???

Allergies      No  Yes
Describe: ___________________________

Sensitivity to numbing medications like Lidocaine?      No  Yes

Diagnosed with coronary artery disease?      No  Yes
Date:____________

Do you know your cholesterol values?      No  Yes
Total-_________ HDL-_________ LDL-_______Triglycerides-_________
Date:____________

Hypertension      No  Yes

What is your current BP?___________________on date:_________________
How long have you had high blood pressure?_____________
Are you taking ANY medications to treat your high blood pressure?      No  Yes
<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong> <em>(Exercise induced?)</em></td>
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<tr>
<td>Inhaler or other treatment for asthma?</td>
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<tr>
<td><strong>Diabetes</strong></td>
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<tr>
<td>Do you know your blood sugar/glucose level?</td>
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<td>Level:________________ ; Date:__________</td>
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<tr>
<td><strong>Blood disorders/wound healing</strong></td>
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<tr>
<td>Do you have any problems with wound healing?</td>
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<tr>
<td>Do you have problems with bleeding or blood clotting?</td>
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<tr>
<td><strong>Other</strong></td>
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<td>Clinical depression</td>
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<td>Nerve or Neurologic disorders</td>
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<td>Any other significant Medical History</td>
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<td>*Describe ____________________________</td>
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<td>*Surgery ____________________________</td>
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<td>Smoking/ Tobacco</td>
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<td>*Ever?</td>
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<td>*How long/quit date</td>
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<tr>
<td>Is your weight unstable?</td>
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<tr>
<td>Do you take any medications</td>
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<tr>
<td>Vitamins</td>
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<td></td>
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<tr>
<td>Statin/cholesterol lowering</td>
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<tr>
<td>ACE inhibitors/water pills</td>
<td></td>
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<tr>
<td>Beta blockers</td>
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<tr>
<td>Asprin or NSAIDS /arthritis meds</td>
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<td></td>
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<tr>
<td>Any hormones or hormone therapy?</td>
<td></td>
<td></td>
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<tr>
<td>Other pain medications</td>
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<tr>
<td>Dietary supplements</td>
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<tr>
<td>Use any inhalers</td>
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<td></td>
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<tr>
<td>Is your weight unstable?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you take any medications</td>
<td></td>
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<tr>
<td>Vitamins</td>
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<td>Statin/cholesterol lowering</td>
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<td>ACE inhibitors/water pills</td>
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<td>Beta blockers</td>
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<td>Asprin or NSAIDS /arthritis meds</td>
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<td>Any hormones or hormone therapy?</td>
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<td>Other pain medications</td>
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<td>Dietary supplements</td>
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<tr>
<td>Use any inhalers</td>
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LITERATURE CITED


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