DISSERTATION

PERIPHERAL BLOOD FLOW REGULATION IN PERSONS WITH MULTIPLE SCLEROSIS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2021

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ABSTRACT

PERIPHERAL BLOOD FLOW REGULATION IN PERSONS WITH MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an inflammatory, degenerative disease of the central nervous system that is believed to be autoimmune in nature. The disease affects approximately one million people in the United States and results in a wide variety of symptoms including impaired physical function, reduced exercise capacity, and increased fatigability. Although considerable effort has been invested in improving our understanding of the neuromuscular contributions to these symptoms, no studies have investigated whether cardiovascular autonomic dysfunction compromises skeletal muscle blood flow in persons with MS (PwMS). Indeed, approximately 50% of PwMS have an abnormal response to tests of cardiovascular autonomic function, and skeletal muscle blood flow is positively associated with exercise capacity. Thus, the overall goal of this dissertation was to determine whether PwMS have impaired skeletal muscle blood flow responses to exercise relative to age- and sex-matched healthy controls.

The primary findings are that 1) the local control of skeletal muscle blood flow during exercise is intact in PwMS, 2) skeletal muscle blood flow is likely reduced during exercise that engages the autonomic nervous system in MS, which may be due to increased α-adrenergic mediated vascular tone, and 3) that PwMS may experience hypersensitivity to α-adrenergic signaling as evidenced by levels of systemic vascular resistance relative to plasma concentrations of norepinephrine. Together, these studies indicate that compromised skeletal muscle blood flow during exercise may contribute to reduced exercise capacity and increased fatigability in PwMS.

ACKNOWLEDGEMENTS

I would like to thank all of the participants who volunteered their time and effort in order to perform these studies. These experiments would not have been possible without the assistance, support, and mentorship of my fantastic lab members including Dr. Janée Terwoord, Nathan Bachman, Brianna Dupre, Meghan Smith, Jacob Anna, and Dr. Nathan Deming. Dr. Christopher Hearon Jr. was instrumental in the development of the overarching hypothesis of this dissertation and data collection in Experiment 1, and I am grateful for the contributions he has made to my professional development. Dr. Thorsten Rudroff gave me the opportunity to become a graduate student in his lab after completing my undergraduate degree, and I am thankful for the unwavering support he provides for his students. This work was made possible by the director of the Human Cardiovascular Physiology Laboratory, Dr. Frank Dinenno, and I am grateful for the guidance and resources he provided. I would also like to acknowledge my committee members, Drs. Brett Fling, Matthew Hickey, Adam Chicco, and Brent Myers. A special thank you to Dr. Brett Fling for his mentorship in the writing of this dissertation and the support that he provides for the students of Health and Exercise Science. I am also appreciative of Dr. Ann Hess, Laurie Biela, and Dr. Kimberly Cox-York for their continual support of my education and professional development.

I cannot thank my friends and family enough for the endless encouragement they provided throughout my time in graduate school. Finally, I would like to thank my wonderful wife Carli for the sacrifices she has made and the unbelievable support, encouragement, and love she has provided throughout this process.

This work was funded by the National Multiple Sclerosis Society grant PP-1805-30884 and NIH/NCATS Colorado CTSA Grant Number UL1 TR002535. Contents are the author's sole responsibility and do not necessarily represent official NIH views.

DEDICATION

This work is dedicated to my parents and grandparents for teaching me the value of an education and the importance of a strong work ethic. I would not be where I am today without the lessons I have learned from each of them.

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CHAPTER 1 – RATIONALE AND EXPERIMENTAL AIMS

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system that is characterized by demyelination throughout the brain and spinal cord. This inflammatory response and subsequent demyelination is believed to be autoimmune in nature, and results in a wide variety of symptoms including sensory and motor dysfunction, neurological pain, autonomic dysfunction such as cardiovascular, sudomotor, bladder and bowel impairments, as well as increased fatigue and exercise intolerance Haensch and Jorg, 2006; Adamec and Habek, 2013; Kister *et al.*, 2013; Langeskov-Christensen *et al.*, 2015). The disease itself manifests in a relapsing-remitting or progressive disease course that can vary widely in severity (Noseworthy *et al.*, 2000). Females are approximately three times more likely to develop MS than males, and an estimated one million people are currently living with the disease in the United States (Wallin *et al.*, 2019).

Although no cure is currently available for MS, corticosteroids and immunomodulators are commonly prescribed to treat and prevent relapses (Noseworthy *et al.*, 2000). Exercise is also now established as an effective means of managing symptomology and improving functional capacity in persons with MS (PwMS) (Motl et al., 2017; Motl & Pilutti, 2012; Motl & Sandroff, 2015; White & Dressendorfer, 2004). However, PwMS continue to engage in less physical activity and have lower exercise capacity than their healthy counterparts (Klaren et al., 2013; Langeskov-Christensen et al., 2015; Motl et al., 2005; Motl & Sandroff, 2015; Sandroff, Dlugonski, et al., 2012). Thus, it is important to understand the factors that may limit exercise tolerance in this clinical population. Neuromuscular aspects of fatigability in MS have been a

significant area of interest due to the high prevalence of fatigue in these individuals (Zijdewind, Prak and Wolkorte, 2016; Severijns *et al.*, 2017), and while the findings have advanced our comprehension of neuromuscular pathophysiology in MS, our understanding of exercise capacity in this clinical group remains limited.

Skeletal muscle blood flow is directly associated with exercise capacity (M J Joyner & Casey, 2015), and it is well-established that reduced oxygen delivery contributes to muscle fatigue (Hepple, 2002; Kent *et al.*, 2016). However, no studies to date have investigated whether MS leads to impaired regulation of blood flow to active skeletal muscle. This review will discuss vascular and autonomic aspects of MS leading to the overarching hypothesis that PwMS have reduced skeletal muscle blood flow responses to exercise compared to a healthy control population.

CARDIOVASCULAR CONTROL DURING EXERCISE

Exercise and activities of daily living require the coordination of many local and systemic responses to ensure adequate oxygen delivery to active tissue while maintaining blood pressure. In short, the cardiovascular system is controlled by the autonomic nervous system during exercise, and autonomic activity is modulated by the interaction between central command, which serves as a feedforward signaling pathway from the brain to increase cardiovascular activity and initiate motor patterns (Mitchell, 1985), and peripheral reflex activity, which largely consists of mechano-, chemo-, and baro- receptors within the active muscle and several cardiovascular centers (Ludbrook and Graham, 1985; Rowell and O'Leary, 1990). These responses are ultimately integrated to increase cardiac output and constrict vascular beds in inactive tissue in order to match oxygen delivery to oxygen demand and maintain mean arterial pressure in the face of robust vasodilation in the active tissue. Several reviews offer extensive

discussion of cardiovascular responses to exercise in humans, as well as the implications of cardiovascular autonomic dysfunction (Florea & Cohn, 2014; Hearon Jr. & Dinenno, 2016; Holwerda et al., 2015; Huang et al., 2015; M J Joyner & Casey, 2015; Michelini et al., 2015; Milazzo et al., 2015; Mortensen & Saltin, 2014). Importantly, PwMS are known to experience cardiovascular autonomic dysfunction as a result of lesions in central cardiovascular control centers (Vita *et al.*, 1993; Saari *et al.*, 2004), and specific examples of such dysfunction are discussed later in this review.

INFLAMMATION AND OXIDATIVE STRESS

Inflammation and oxidative stress are key contributors to the pathophysiology of MS, and they are generally elevated in this disease population (Ibitoye et al., 2016; Kallaur et al., 2017; S. R. Oliveira et al., 2012; Patejdl et al., 2016). Importantly, the negative impact of inflammation and oxidative stress on nitric oxide bioavailability and vascular function is well established (el Assar et al., 2013; Rodriguez-Manas et al., 2009). Interventional studies have demonstrated that acute administration of the potent antioxidant, ascorbic acid (Vitamin C), reverses the age-related impairment in skeletal muscle blood flow during exercise (Kirby *et al.*, 2009; Richards *et al.*, 2015). These findings not only provide further evidence of the detrimental effect of oxidative stress on vascular function during dynamic exercise, but also highlight the importance of investigating vascular control in populations with elevated inflammation and oxidative stress.

Flow-mediated dilation is a common means of noninvasively assessing vascular function by measuring dilation in the brachial artery after a brief period of ischemia. Importantly, this response has a strong reliance on nitric oxide and is predictive of future cardiovascular disease risk (Mitchell *et al.*, 2004; Yeboah *et al.*, 2009; Green *et al.*, 2014). The blood flow response following forearm ischemia, termed reactive hyperemia, is the stimulus for flow-mediated

dilation and is an additional measure of vascular function that is predictive of cardiovascular disease risk (Philpott *et al.*, 2009; Anderson *et al.*, 2011). While absolute levels of peak reactive hyperemia may be reduced in PwMS (Ranadive *et al.*, 2012), conflicting data indicates that reactive hyperemia and flow-mediated dilation are not altered in these individuals (Fjeldstad *et al.*, 2011). Larger studies are likely necessary to determine whether vascular function is impaired in MS, and to further investigate findings which indicate peripheral levels of inflammation and oxidative stress are not always elevated when PwMS are in remission (Giovannoni *et al.*, 2001; Soilu-Hänninen *et al.*, 2005; Fjeldstad *et al.*, 2011).

ENDOTHELIN-1

Endothelin-1 (ET-1) is a potent vasoconstrictor that is derived from the vascular endothelium and is known to play a key role in age-associated vascular dysfunction (Thijssen et al., 2007; van Guilder et al., 2007; Westby et al., 2011) and essential hypertension (Cardillo *et al.*, 1999; Taddei *et al.*, 1999; Bruno *et al.*, 2011). Several studies have identified increased plasma concentrations of ET-1 in the peripheral and central circulation of PwMS (Haufschild *et al.*, 2001; D'Haeseleer *et al.*, 2013), indicating that this clinical population may experience increased vasoconstrictor tone. This hypothesis is supported by key findings which demonstrated that MS-associated cerebral hypoperfusion is abolished following the administration of the non-specific ET-1 antagonist, bosentan (D'Haeseleer *et al.*, 2013). There is also evidence to indicate that ET-1 release increases during exercise (Barrett-O'Keefe *et al.*, 2013), and previous work established the role of ET-1 in the exercise-induced redistribution of blood flow away from the splanchnic circulation (Maeda *et al.*, 2002). Moreover, endogenous ET-1 can restrain blood flow to active tissue and contribute to the preservation of mean arterial pressure during exercise in healthy adults (Barrett-O'Keefe *et al.*, 2013). Although not all

studies indicate that ET-1 is elevated peripherally in MS (Jankowska-Lech *et al.*, 2015), the body of evidence to date suggests that PwMS may experience increased ET-1-mediated vasoconstriction. However, the potential implications of augmented ET-1 signaling in MS have yet to be considered in the context of exercise.

CARDIOVASCULAR AUTONOMIC FUNCTION

Cardiovascular autonomic function is assessed using a variety of tests that are designed to challenge cardiovascular reflexes. Previous studies utilizing tests such as sustained handgrip, breathing maneuvers, and orthostatic challenges indicate that PwMS are susceptible to cardiovascular autonomic dysfunction (Nasseri *et al.*, 1998; Flachenecker *et al.*, 1999, 2003). While the reported prevalence of this dysfunction ranges from 7% to 60%, it is commonly accepted that PwMS experience cardiovascular autonomic dysfunction as indicated by abnormal heart rate and blood pressure responses, including reduced heart rate variability, postural orthostatic tachycardia syndrome, and orthostatic hypotension in response to standardized tests (Haensch and Jorg, 2006; Huang, Jay and Davis, 2015). More information on autonomic dysfunction in multiple sclerosis, including in-depth discussions of abnormal cardiovascular autonomic responses, can be found in reviews by Adamec and Habek, and Haensch and Jörg (Haensch and Jorg, 2006; Adamec and Habek, 2013).

Although few studies are available, the current evidence indicates that PwMS also experience blunted cardiovascular responses during exercise. Peak heart rate and systolic blood pressure were each attenuated following a graded cycle exercise task in PwMS compared to healthy individuals (Cohen et al., 2016). Moreover, Senaratne and colleagues found evidence of reduced heart rate and blood pressure responses to arm ergometry exercise in several PwMS (Senaratne *et al.*, 1984). The findings of each of these studies indicate that PwMS may

experience reduced perfusion pressure during exercise, ultimately resulting in decreased skeletal muscle blood flow. Huang and colleagues provide an excellent review of the current literature on cardiovascular and thermoregulatory autonomic dysfunction in MS and their potential implications in exercise tolerance (Huang, Jay and Davis, 2015).

Sympathetic nerve activity (SNA) results in the release of norepinephrine from sympathetic nerve terminals, which then binds to postjunctional α-adrenergic receptors on the vascular smooth muscle to elicit vasoconstriction (Piascik *et al.*, 1996). While no measures of SNA have been taken during exercise in PwMS, key findings by Keller and colleagues indicate that muscle SNA and subsequent plasma norepinephrine concentrations are reduced in these individuals at rest (Keller *et al.*, 2014). Importantly, chronically reduced SNA, which is known to occur in individuals with autonomic failure and neuropathy, is associated with hypersensitivity to α-adrenergic stimulation (Bannister *et al.*, 1979; Biaggioni, Robertson and Robertson, 1994; Dejgaard *et al.*, 1996). Indeed, PwMS demonstrated robust peripheral vasoconstriction in response to activation of the muscle metaboreflex, which was achieved via post-exercise muscle ischemia (Marongiu et al., 2015). Although no measures of SNA or plasma norepinephrine were taken in the participants, these data combined with previous findings demonstrating reduced SNA at rest indicate that PwMS may experience hypersensitivity to plasma norepinephrine. However, future studies are needed to better understand sympathetic control of the vasculature during exercise in MS.

PERSPECTIVES AND FUTURE DIRECTIONS

The cumulative evidence to date supports the hypothesis that PwMS may experience vascular dysfunction as a result of increased inflammation, oxidative stress, ET-1, and hypersensitivity to plasma norepinephrine. Moreover, blunted heart rate and blood pressure

responses to exercise may compromise perfusion pressure. Taken together, these studies suggest impaired regulation of skeletal muscle blood flow may limit exercise hyperemia, providing novel insights to a potential underlying cause of exercise intolerance in this clinical population.

Research on skeletal muscle blood flow in MS is currently in its infancy, and descriptive studies are first needed to determine whether hyperemic responses to exercise stimuli are impaired in PwMS. Given the potential for alterations in basal levels of vasoactive substances and autonomic activity to affect skeletal muscle blood flow responses to exercise in individuals with MS, studies should be carefully designed to differentiate these factors. The rhythmic handgrip exercise model offers an established approach to investigate hemodynamic responses to exercise independent of changes in SNA, heart rate, or mean arterial pressure when performed at mild-to-moderate intensities for relatively short periods of time (Victor and Seals, 1989; Batman et al., 1994; Kirby et al., 2009). As a result, this approach allows investigators to determine the effect of systemically circulating factors, as well as locally produced vasoactive substances, on the control of skeletal muscle blood flow during exercise. Studies utilizing the handgrip exercise model with concomitant plasma measures of inflammation, oxidative stress, ET-1, and norepinephrine may provide key evidence in differentiating local and autonomic control of skeletal muscle blood flow in PwMS.

The single-leg kicking model was first developed by Andersen and Saltin in 1985 in their seminal work that aimed to quantify maximal perfusion of skeletal muscle in humans (Andersen and Saltin, 1985). This exercise modality was particularly effective in achieving the aims of this study due to the active muscle mass being large enough to significantly increase cardiac output and mean arterial pressure, while remaining small enough to not outstrip maximal cardiac output. As a result, the single-leg kicking model continues to be utilized by exercise physiologists

aiming to better understand the control of skeletal muscle blood flow during exercise, and the advent of Doppler ultrasound now permits non-invasive measures of blood flow in conjunction with this model (Donato et al., 2006; Wray et al., 2007; Mortensen et al., 2012; Barrett-O'Keefe et al., 2013).

Given the known cardiovascular autonomic dysfunction in MS discussed above, future studies should utilize the single-leg kicking model to assess skeletal muscle blood flow during exercise that engages the autonomic nervous system. These studies will be critically important in furthering our understanding of skeletal muscle blood flow control in MS due to the integration of autonomic signaling to redistribute blood flow to active tissue while maintaining blood pressure during the kicking exercise. Secondary measures including cardiac output, mean arterial pressure, and plasma concentrations of norepinephrine and ET-1 should also be measured at several intensities due to evidence indicating that the signaling pathways for each of these vasoactive substances may be affected in MS (D'Haeseleer et al., 2013; Keller et al., 2014; Huang, Jay and Davis, 2015; Marongiu et al., 2015).

The metaboreflex test offers a controlled manner in which to increase SNA, and previous findings indicate that PwMS may respond differently to this stimulus. Although the MS cohort achieved a normal pressor response, they accomplished this increased mean arterial pressure via robust peripheral resistance as opposed to the expected increase in cardiac output (Marongiu et al., 2015). There is also data to suggest that PwMS experience adrenergic hyperactivity, which is associated with increased blood pressure (Habek, Mutak, et al., 2020; Habek, Pucić, et al., 2020). Alternatively, Huang and colleagues utilized controlled neck pressure to simulate carotid hypotension and ultimately increase SNA (Huang et al., 2016). The PwMS demonstrated a blunted pressor response, which was likely due to a diminished reduction in total vascular

conductance relative to the control participants. The conflicting data above highlights the need for future work aiming to better understand autonomic regulation of SNA in PwMS. While the discrepancies in the findings between these papers may be due to the differing stimuli, it is clear that MS influences sympathetic signaling. Future studies measuring end-organ responsiveness in combination with indices of SNA are necessary to better understand sympathetic control of the vasculature in PwMS.

Previous studies investigating vascular and autonomic function in MS have laid the foundation for an exciting opportunity to better understand the control of exercise hyperemia in these individuals. Although our knowledge is currently limited, carefully designed studies aimed at characterizing skeletal muscle blood flow responses to exercise in MS hold the potential to have a significant impact on this growing field of research. The cumulative findings will help guide future work with the goal of increasing exercise tolerance, reducing fatigue, and ultimately improving quality of life for those living with MS.

SPECIFIC AIMS

Experiment 1: To determine whether local control of exercise hyperemia is impaired in PwMS relative to healthy control participants

Experiment 2: a) To determine whether PwMS have impaired hyperemic responses to exercise that engages the autonomic nervous system.

b) To quantify plasma norepinephrine, ET-1, inflammation, and oxidative stress at rest, and to measure the release of norepinephrine and ET-1 during exercise in PwMS and healthy controls.

Experiment 3: To assess the metaboreflex in combination with measures of plasma norepinephrine in PwMS and healthy controls.

CHAPTER 2 – MANUSCRIPT 1

Local control of exercise hyperemia is preserved in persons with multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system that is autoimmune in nature. Nearly one million people are estimated to be living with MS in the United States, and females are approximately three times more likely to develop the disease than males (Wallin et al., 2019). While the pathophysiology of MS is not fully elucidated, it is largely accepted that the symptoms of the disease result from demyelination within the central nervous system (Noseworthy et al., 2000). This neurological damage leads to a wide variety of symptoms including reduced mobility, spasticity, fatigue, pain, bladder and bowel dysfunction, and sensory impairments (Kister et al., 2013).

Although persons with MS (PwMS) were historically discouraged from engaging in physical exercise out of fear that it would worsen their symptoms and to preserve their energy, exercise is now widely accepted as an effective means of reducing symptomology (Motl et al., 2017; Motl & Pilutti, 2012; Motl & Sandroff, 2015; White & Dressendorfer, 2004). Despite the known benefits of exercise for PwMS, these individuals are less active and have reduced exercise capacity relative to their healthy counterparts (Klaren et al., 2013; Langeskov-Christensen et al., 2015; Motl et al., 2005, 2015; Sandroff, Dlugonski, et al., 2012). Although several studies have investigated the underlying cause of reduced exercise capacity and increased fatigability in PwMS, these studies have largely focused on neuromuscular dysfunction (Zijdewind, Prak and Wolkorte, 2016; Severijns et al., 2017). While this is a logical approach

considering the neurological origins of the disease, no studies to date have considered reduced skeletal muscle blood flow and subsequent oxygen delivery as a potential contributor to impaired exercise capacity in PwMS.

The matching of oxygen delivery to oxygen demand within active skeletal muscle is a core principle of cardiovascular physiology that must be maintained to avoid premature fatigue of the muscle. As a result, the human cardiovascular system consists of a multitude of signaling pathways with substantial redundancy in order to achieve the well-established linear correlation between exercise workload and blood flow to the active limb(s) (M J Joyner & Casey, 2015). Alterations in the net integration of these signals, which occur with aging and many disease states, is associated with reduced skeletal muscle blood flow and oxygen delivery during exercise (Kingwell et al., 2003; Hearon Jr. and Dinenno, 2016; Iepsen et al., 2017), ultimately reducing exercise capacity. Importantly, several studies have measured reduced levels of cerebral blood flow in PwMS when compared to healthy controls (D'Haeseleer et al., 2013, 2015; Debernard et al., 2014; Hojjat et al., 2016; Messinis et al., 2019). Although the underlying causes of this impairment are largely unexplored, D'Haeseleer and colleagues present strong evidence to suggest that the potent vasoconstrictor, endothelin-1 (ET-1), is a key contributing factor (D'Haeseleer et al., 2013). The authors built on previous data demonstrating elevated plasma concentrations of ET-1 in PwMS (Haufschild et al., 2001) by exploring the effects of the non-specific ET-1 antagonist, bosentan, on cerebral blood flow. Indeed, the decrement in cerebral blood flow initially observed in the PwMS was abolished following systemic administration of bosentan, suggesting an ET-1-mediated restriction of cerebral blood flow in MS (D'Haeseleer et al., 2013). However, the effects of ET-1 on peripheral blood flow regulation in PwMS have not been investigated.

Inflammation and oxidative stress play a key role in the pathophysiology of MS (Oliveira et al., 2012; Ibitoye et al., 2016; Patejdl et al., 2016; Kallaur et al., 2017), and they are well-established as detrimental factors leading to impaired blood flow regulation in disease states, including aging (Rodriguez-Manas et al., 2009; el Assar, Angulo and Rodriguez-Manas, 2013). Local and systemic doses of the potent antioxidant, ascorbic acid (Vitamin C), have been utilized to acutely reverse age-related impairments in skeletal muscle blood flow during exercise (Michael J. Joyner, 2009; Kirby et al., 2009). Therefore, inflammation and oxidative stress may negatively affect vascular function and blood flow regulation in PwMS.

The purpose of this study was to determine whether regulation of skeletal muscle blood flow is impaired in PwMS during exercise. We utilized the handgrip exercise model as a means of assessing potential differences in local vascular signaling independent of physiologically significant changes in autonomic responses including heart rate and blood pressure. Given the known influence of elevated plasma ET-1 in PwMS, in addition to systemically elevated levels of inflammation and oxidative stress, we hypothesized that PwMS would have reduced skeletal muscle blood flow during graded handgrip exercise relative to age-matched healthy controls.

METHODS

The study protocol was completed over the course of two visits to the Human Performance Clinical Research Laboratory on the Colorado State University campus. All procedures were approved by the Colorado State University Institutional Review Board and were conducted in accordance with the Declaration of Helsinki. Seven healthy control participants (5F, 2M) and 9 PwMS (7F, 2M) completed each visit after providing written, informed consent, and following a fasting period of at least 4 hrs. All participants were non-smokers, sedentary to moderately physically active, and not taking medications to control blood pressure or cholesterol.

Participants were required to not have been hospitalized or had any changes to their medications within 3 months of starting the study, and the PwMS were free of relapses for at least 3 months prior to the study. All experiments were performed in a temperature-controlled laboratory (20 - 22°C).

Visit 1

Disability Status

Disability status of the MS cohort was determined using the Patient Determined Disease Steps (PDDS) questionnaire (Hohol, Orav and Weiner, 1995). This questionnaire was developed as an alternative to the Expanded Disability Status Scale, which is a physician-administered neurological exam. PDDS questionnaire scores are linearly and strongly associated with disability status determined using the Expanded Disability Status Scale (Hohol, Orav and Weiner, 1995; Learmonth et al., 2013).

Body Composition and Forearm Fat-Free Mass

A whole-body Dual Energy X-Ray Absorptiometry (Hologic, Inc., Bedford, MA, USA) scan was performed on each participant to measure body composition. A region of interest was then drawn around the non-dominant forearm, defined as the proximal to distal radio-ulnar joint. Forearm fat-free mass (FFM) was calculated using the equation:

$$Forearm\ FFM\ (dL) = \frac{Forearm\ Lean\ Mass\ (g)}{1.1\ \left(\frac{g}{mL}\right)}\ x\ 100^{-1}$$

Maximal Hand Grip Strength

Maximal voluntary hand grip strength of the non-dominant forearm was assessed using a hydraulic JAMAR 5030J1 hand dynamometer (Sammons Preston Rolyan, Bolingbrook, IL). The dynamometer was adjusted for each participant to control for hand size, and strong verbal encouragement was given while the participant performed a series of maximal grip strength

measurements. The strongest trial was used to calculate workloads for the graded hand grip exercise trial performed during the second visit.

Peak Forearm Work Rate

Exercise capacity of the experimental forearm was determined using a maximal work rate test, during which participants performed dynamic, graded hand grip exercise at a duty cycle of 1 s contraction : 2 s relaxation. The weight was lifted 4-5 cm over the pulley system, and audio and visual cues were used to ensure proper timing of the contractions (Terwoord et al., 2020). The test began with 5 lbs and was increased by 2.5 lbs each min until the participant was no longer able to complete a properly timed, full contraction (Richards et al., 2014). The workload during the last successfully completed trial was considered the peak work rate (WRpeak).

Visit 2

Rhythmic Handgrip Exercise

Participants performed rhythmic handgrip exercise in the manner described above at 5%, 15%, and 25% of their maximal grip strength. Contractions at each intensity were performed for approximately 3 min, and a fan was pointed toward the exercising forearm to minimize the contribution of skin blood flow to overall forearm hemodynamics.

Forearm Blood Flow and Vascular Conductance

Brachial artery mean blood velocity (MBV) and diameter were measured on the experimental arm with a 12 MHz linear array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA). Brachial artery diameter measures were made in triplicate at end diastole during steady state conditions using images that were acquired in duplex mode. Blood velocity measures were performed using a probe insonation angle < 60° and a frequency of 5MHz. MBV was calculated as a weighted mean of the spectrum of Doppler shift frequencies

analyzed via a Multigon 500 M TCD spectral analyzer (Multigon Industries, Mount Vernon, NY, USA). Forearm blood flow (FBF) and forearm vascular conductance (FVC) were normalized to forearm FFM and calculated using the equations:

$$FBF\ \left(\frac{mL}{min/Forearm\ FFM(dL)}\right)$$

$$= MBV\ \left(\frac{cm}{s}\right) x\ \pi\ \left(\frac{Brachial\ Artery\ Diameter\ (cm)}{2}\right)^2\ x \frac{60\ s}{min}\ x\ Forearm\ FFM^{-1}$$

$$FVC\ \left(\frac{mL}{min/100\ mmHg/Forearm\ FFM(dL)}\right)$$

$$= \frac{FBF\ \left(\frac{mL}{min}\right)}{Mean\ Arterial\ Pressure\ (mmHg)} x\ 100\ x\ Forearm\ FFM^{-1}$$

Heart Rate and Blood Pressure

Heart rate (HR) was calculated using a 3-lead electrocardiogram and mean arterial blood pressure (MAP) was non-invasively measured on a beat-by-beat basis using a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) (Chin and Panerai, 2012).

Hemodynamic Data Acquisition and Analysis

Data were collected at 250 Hz and analyzed offline using data acquisition and signal-processing software (WinDaq; DATAQ Instruments, Akon, OH, USA). Baseline and steady-state FBF, MAP, FVC, and HR were calculated over 30 s averages at rest and steady-state for each exercise intensity.

Actigraphy Measures

Total physical activity was objectively measured using ActiGraph GT3X+ accelerometers (Pensacola, FL, USA) that were worn on the self-reported non-dominant hip for 7-10 days between the first and second visit. Data were collected at 30 Hz and accelerometer

counts in the vertical axis were analyzed in counts per min using 15 s epochs (Sandroff et al., 2012). A valid day was defined as a minimum of 10 hrs of wear time, and a minimum of 4 valid days with 1 weekend day was used for the analysis (Troiano et al., 2008). Total physical activity per day was calculated as the sum of time spent in moderate-vigorous physical activity and light physical activity divided by the number of valid wear days. Wear logs were completed by each participant to verify the accelerometer data.

Statistical Analysis

Normality of participant characteristics and baseline hemodynamics was determined using the Shapiro Wilk test. Group differences were assessed using T-Tests and Wilcoxon Signed Rank Tests for parametric and non-parametric data, respectively. Cohen's D effect size was calculated for group comparisons of baseline hemodynamics and interpreted as small (d = 0.2), medium (d = 0.5) or large (d = 0.8) (J. Cohen, 1988). Exercise hemodynamic responses were analyzed using two-way repeated measures analysis of covariance (Group x Intensity) with baseline values included in the model as a covariate. Residual diagnostic plots were used to assess model assumptions. FBF and FVC models displayed evidence of unequal variance and were log transformed to satisfy model assumptions. In order to further interpret group differences, Eta-squared effect size (η^2) was calculated for the main effects and the Group x Intensity interaction. η^2 was interpreted as small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), or large ($\eta^2 = 0.14$) (J. Cohen, 1988). All data were analyzed in R (R Core Team, 2021) and significance was determined using an alpha of 0.05.

RESULTS

Participant Characteristics

Participant characteristics are presented in Table 2.1. The two groups were successfully matched for age, body mass, height, and body mass index. Handgrip strength and WRpeak, as well as daily physical activity, were also matched between groups. The MS cohort had a PDDS range of 1-3 and a disease duration of 14.8 ± 8.2 years.

Table 2.1: Participant characteristics

	CTRL	*MS*
n	7 (5F, 2M)	9 (7F, 2M)
Age, y	52.7 ± 10.0	54.7 ± 8.9
Body Mass, kg	63.4 ± 16.0	62.9 ± 9.1
Height, cm	166.0 ± 11.8	167.4 ± 9.9
BMI, kg/m^2	22.8 ± 3.2	22.5 ± 2.4
Non-Dominant MVC, N	208.1 ± 54.3	209.6 ± 32.3
Non-Dominant WRpeak, lbs	23.9 ± 7.8	22.8 ± 5.3
Physical Activity, min/day	186.0 ± 49.4	159.0 ± 34.5
Disability Status, PDDS	-	1-3
Disease Duration, y	-	14.8 ± 8.2

Values are mean ± SD. Physical activity calculated from the sum of time spent in light and moderate activity per day based on actigraphy. MVC, maximal voluntary contraction; WRpeak, peak work-rate; PDDS, patient determined disease steps questionnaire. $P > 0.05$ for all group comparisons.

Baseline Hemodynamics

Baseline hemodynamics are presented in Table 2.2. The PwMS had a higher HR, MAP, and FBF at rest compared to the control participants. Although not significant, there was a medium effect size for increased baseline FVC in the MS cohort.

Exercise Hemodynamics

There were no group differences in the HR or MAP response to exercise (Figure 2.1; Tables 2.3 and 2.4). FBF and FVC were also matched between the groups across the three exercise intensities (Figure 2.2; Tables 2.3 and 2.4).

Table 2.2: Baseline hemodynamics

	CTRL	*MS*	*P-Value*	*Cohen's D*
HR, beats/min	49 ± 3	59 ± 8	0.01*	-1.57
MAP, mmHg	84.4 ± 9.0	96.6 ± 11.0	0.03*	-1.20
FBF, ml/min/dl FFM	3.8 ± 1.2	5.6 ± 2.4	0.03*	-1.18
FVC, ml/min/100 mmHg/ dl FFM	4.9 ± 1.6	6.1 ± 3.3	0.30	-0.72

HR, heart rate; MAP, mean arterial pressure; FBF, forearm blood flow; FFM, fat-free mass; FVC, forearm vascular conductance. * $P < 0.05$.

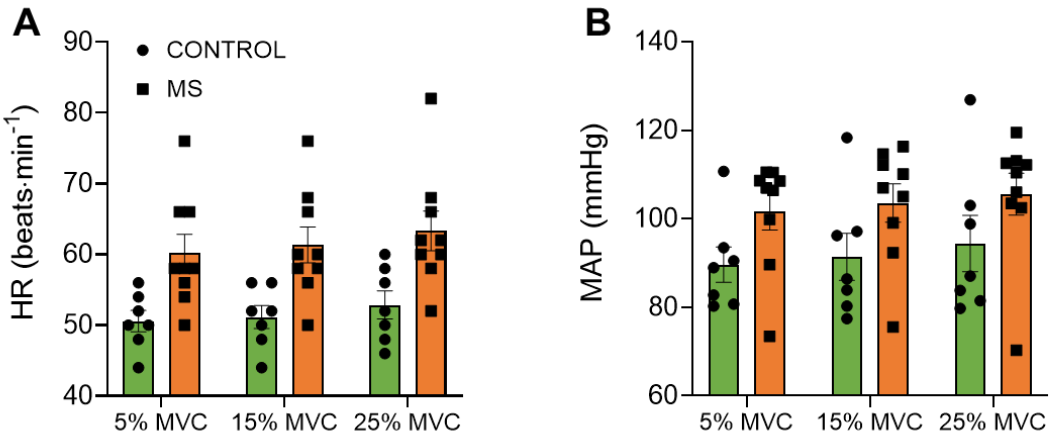

Figure 2.1: Heart rate and blood pressure responses to graded handgrip exercise

Group and individual data showing heart rate (A) and blood pressure (B) responses to graded handgrip exercise in healthy controls and participants with multiple sclerosis. HR, heart rate; MAP, mean arterial pressure; MS, multiple sclerosis; MVC, maximal voluntary contraction. $P > 0.05$ for all group comparisons.

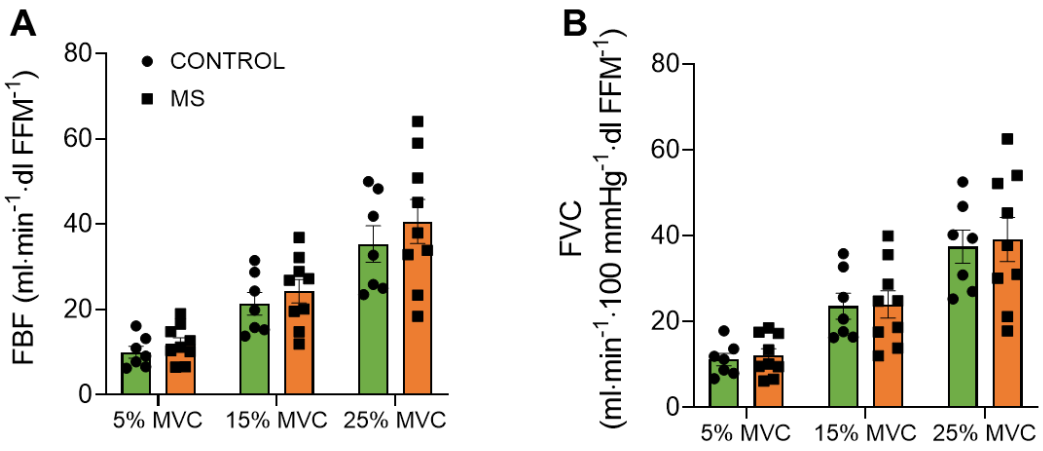

Figure 2.2: Blood flow and vascular conductance responses to graded handgrip exercise

Group and individual data showing blood flow (A) and vascular conductance (B) responses to graded handgrip exercise in healthy controls and participants with multiple sclerosis. FBF, forearm blood flow; FVC, forearm vascular conductance; MS, multiple sclerosis; MVC, maximal voluntary contraction. $P > 0.05$ for all group comparisons.

Table 2.3: Exercise response statistics

	P-Value	*Eta-Squared*
HR		
Group	0.01	0.40
Intensity	< 0.01	0.63
Group x Intensity	0.73	0.05
MAP		
Group	0.10	0.18
Intensity	0.05	0.37
Group x Intensity	0.87	0.02
FBF		
Group	0.39	0.05
Intensity	< 0.001	0.99
Group x Intensity	0.92	0.01
FVC		
Group	0.82	< 0.01
Intensity	< 0.001	0.98
Group x Intensity	0.93	< 0.01

HR, heart rate; MAP, mean arterial pressure; FBF, forearm blood flow; FVC, forearm vascular conductance

Table 2.4: Absolute values

	Control	MS	
	Mean ± SD	*Mean ± SD*	*P-Value*
HR			
5% MVC	51 ± 4	60 ± 8	0.90
15% MVC	51 ± 4	61 ± 8	0.69
25% MVC	53 ± 5	63 ± 8	0.59
MAP			
5% MVC	89.6 ± 10.6	101.6 ± 12.5	0.60
15% MVC	91.4 ± 14.0	103.6 ± 13.0	0.63
25% MVC	94.4 ± 16.8	105.6 ± 14.3	0.47
FBF			
5% MVC	10.0 ± 3.7	12.0 ± 4.3	0.37
15% MVC	21.3 ± 7.0	24.3 ± 8.2	0.32
25% MVC	35.5 ± 11.3	40.6 ± 15.5	0.29
FVC			
5% MVC	11.1 ± 3.8	12.0 ± 4.8	0.16
15% MVC	23.6 ± 8.0	24.0 ± 9.5	0.12
25% MVC	37.4 ± 10.2	39.1 ± 15.4	0.12

P-values are pairwise comparisons between groups at each intensity while controlling for baseline values of each measure. HR, heart rate; MAP, mean arterial pressure; FBF, forearm blood flow; FVC, forearm vascular conductance; MVC, maximal voluntary contraction

DISCUSSION

The purpose of this study was to determine whether MS negatively impacts local control of exercise hyperemia. We tested the hypothesis that PwMS have reduced skeletal muscle blood flow during exercise relative to age-matched control individuals by measuring blood flow responses during a graded handgrip exercise task. These data represent the first measures of exercise hyperemia in PwMS, and they indicate that MS does not impair local control of skeletal muscle blood flow during exercise.

Local Control of Skeletal Muscle Blood Flow

The handgrip exercise model is established as an effective means of assessing the control of skeletal muscle blood flow independent of physiologically meaningful changes in HR, MAP,

or sympathetic nerve activity (Victor and Seals, 1989; Batman et al., 1994; Kirby et al., 2009). As a result, this model is used to assess the influence of systemically circulating compounds that are not believed to change due to the exercise stimulus, as well as the local release of vasoactive molecules within the active tissue. As expected, there were no differences in HR or MAP between the groups during the graded handgrip exercise task (Figure 2.1). However, we did not observe any differences in FBF or local vascular tone, as indicated by FVC, in response to the exercise stimulus (Figure 2.2; Tables 2.3 and 2.4). These findings suggest that vascular function is likely normal in mild-to-moderately disabled PwMS.

Vascular Function in MS

Previous studies have assessed vascular function in MS using traditional measures of flow-mediated dilation and reactive hyperemia. Although no group differences were identified in flow-mediated dilation (Fjeldstad et al., 2011), a separate study measured a significantly lower reactive hyperemia response in the PwMS (Ranadive et al., 2012). However, this finding was no longer significant when group differences in physical activity were accounted for in the model. These data indicate that vascular dysfunction may occur secondary to reductions in physical activity in PwMS. We did not identify a significant difference in physical activity between our groups, and we speculate that similar activity levels contributed to matched hyperemic responses between the two groups.

Limitations and Future Directions

Due to financial constraints associated with this pilot study, we were not able to assess plasma concentrations of vasoactive compounds that are potentially altered with MS. Previous data demonstrating increased levels of ET-1, inflammation, and oxidative stress in PwMS were influential in the development of the hypothesis in this study. Unfortunately, MS is a

heterogenous disease and there is evidence to suggest that these compounds are not elevated in all individuals (Giovannoni et al., 2001; Soilu-Hänninen et al., 2005; Fjeldstad et al., 2011; Jankowska-Lech et al., 2015). Thus, it is critical to assess plasma markers of candidate vasoactive compounds in conjunction with measures of exercise hyperemia to better understand the control of skeletal muscle blood flow in MS.

The handgrip model utilized in this study was selected to intentionally minimize the influence of autonomic nervous system activity. However, small muscle mass handgrip exercise is not representative of whole-body physical activity. Traditional forms of exercise and activities of daily living require the integration of systemic signaling pathways to properly distribute blood flow and maintain MAP. Current evidence indicates that PwMS have impaired cardiovascular autonomic responses to exercise (Huang, Jay and Davis, 2015), and future studies are needed to determine whether this dysfunction is detrimental to exercise hyperemia in these individuals. Moreover, the lower extremities are typically more affected by MS than the upper extremities (Schwid et al., 1999), and assessments of the upper extremities may not appropriately differentiate PwMS from controls. However, it is important to note that our hypothesis was formed on the premise that increased levels of systemically circulating vasoactive compounds would negatively affect the blood flow response to exercise, and there is no reason to believe that the concentration of these compounds would differ between the upper and lower extremities.

Perspectives and Conclusion

MS is a debilitating disease of the central nervous system that greatly reduces functional capacity and quality of life (Kobelt et al., 2006; Campbell et al., 2014; Kjolhede et al., 2015; Jensen et al., 2016). Increased physical activity is positively associated with quality of life in these individuals, independent of disability status (Marck et al., 2014). Thus, it is important to

understand the factors that limit an active lifestyle for PwMS. Compromised regulation of skeletal muscle blood flow occurs in several clinical populations including aging, heart failure, diabetes mellitus, and chronic obstructive pulmonary disorder (Menon et al., 1992; Dinenno et al., 1999; Lalande et al., 2008; Kirby et al., 2009; Barrett-O et al., 2014; Hearon Jr. and Dinenno, 2016; Oliveira et al., 2016; Iepsen et al., 2017). Although the findings of the current study suggest that MS does not impair skeletal muscle blood flow during handgrip exercise, cardiovascular autonomic dysfunction may be detrimental to exercise hyperemia during larger muscle mass exercise.

CHAPTER 3 – MANUSCRIPT 2

Reduced skeletal muscle blood flow during exercise in persons with multiple sclerosis

INTRODUCTION

The onset of exercise or activities of daily living initiates robust local vasodilation in the active tissue and increased autonomic activity to ensure oxygen demand is met in contracting skeletal muscle. Although the factors that control the local dilatory response to exercise are not fully understood, there are many putative compounds including nitric oxide, prostaglandins, potassium, and adenosine triphosphate, all of which likely contribute to vasodilation in a highly redundant manner (Joyner & Casey, 2015). Additionally, it is important to consider the effect of exercise-induced augmentation of constrictor substances such as endothelin-1 (ET-1), which is known to increase during exercise (Wray et al., 2007; Barrett-O'Keefe et al., 2013). Indeed, previous data indicate that ET-1 restricts blood flow to active tissue during exercise in healthy humans (Barrett-O'Keefe et al., 2013), and likely contributes to reduced exercise hyperemia in clinical populations with increased plasma ET-1 (Iepsen et al., 2017).

Modulation of autonomic activity during exercise requires precise coordination of central command and afferent signaling from mechano-, chemo-, and baro- receptors (Ludbrook & Graham, 1985; Mitchell, 1985; Rowell & O'Leary, 1990). The subsequent response includes parasympathetic withdrawal and increased sympathetic nerve activity (SNA), which aids in augmenting cardiac output and constricting blood vessels of inactive skeletal muscle and the splanchnic circulation to redistribute blood flow to active tissue. Importantly, blood pressure in individuals with autonomic dysfunction may actually fall during exercise, and this is

hypothesized to be caused by inadequate constriction of inactive vascular beds (Marshall, Schirger and Sheperd, 1961).

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system that causes neural damage throughout the brain and spinal cord. Persons with MS (PwMS) suffer from a wide variety of symptoms that greatly reduce quality of life including exercise intolerance, increased fatigability, and cardiovascular autonomic dysfunction (Senaratne et al., 1984; Flachenecker et al., 1999; de Haan et al., 2000; Adamec and Habek, 2013; Huang, Jay and Davis, 2015; Langeskov-Christensen et al., 2015; Zijdewind, Prak and Wolkorte, 2016). In addition to reduced heart rate (HR) and blood pressure responses to exercise (Cohen et al., 1989; Senaratne et al., 1984), direct measures of muscle SNA and plasma norepinephrine concentrations demonstrate that PwMS may also experience lower levels of SNA at rest (Keller et al., 2014). Moreover, elevated plasma ET-1 is known to restrict cerebral perfusion in PwMS (D'Haeseleer et al., 2013) but its effects on the peripheral circulation have not been investigated.

These data indicate that neural damage in MS may inhibit SNA, which compromises HR and blood pressure responses to exercise. Additionally, increased levels of ET-1 may lead to enhanced vasoconstrictor tone within active muscle. Together, PwMS may suffer from reduced perfusion pressure and exaggerated vascular tone in the active tissue, ultimately limiting blood flow. Therefore, the purpose of this study was to determine whether PwMS experience reduced skeletal muscle blood flow during an exercise task that engages the autonomic nervous system. In an effort to better understand the factors controlling skeletal muscle blood flow in PwMS, we also aimed to assess plasma concentrations of norepinephrine and ET-1 for the first time in PwMS during exercise. Finally, we chose to measure markers of inflammation and oxidative stress at rest in each group to further verify the findings of our previous findings using the

handgrip exercise model. We hypothesized that PwMS would have reduced skeletal muscle blood flow during exercise relative to a group of age- and sex-matched healthy control participants due to compromised SNA and enhanced vascular tone via increased ET-1. We also hypothesized that inflammation and oxidative stress would not differ between the two groups.

METHODS

The data was collected over the course of two visits to the Human Performance Clinical Research Laboratory on the Colorado State University campus. All procedures were approved by the Colorado State University Institutional Review Board and were conducted in accordance with the Declaration of Helsinki. Eight healthy control participants (7F, 1M) and 8 PwMS (7F, 1M) completed each visit after providing written, informed consent, and following an overnight fast. All participants were non-smokers, sedentary to moderately physically active, and not taking medications to control blood pressure or cholesterol. Participants were required to not have been hospitalized or had any changes to their medications within 3 months of starting the study, and all participants in the MS cohort were free of relapses for at least 3 months prior to the study. All experiments were performed in a temperature-controlled laboratory (20 - 22°C).

Visit 1

Questionnaires

Disability status of the PwMS was determined using the Patient Determined Disease Steps (PDDS) questionnaire (Hohol, Orav and Weiner, 1995). This questionnaire was developed as an alternative to the Expanded Disability Status Scale, which is a physician-administered neurological exam. PDDS questionnaire scores are linearly and strongly associated with disability status determined using the Expanded Disability Status Scale (Hohol, Orav and Weiner, 1995; Learmonth et al., 2013).

Activity levels of each participant were assessed using the International Physical Activity Questionnaire. This questionnaire has been validated in the general population (Craig et al., 2003) and in PwMS (Gosney et al., 2007; Motl et al., 2006). Total physical activity was quantified as metabolic equivalent min per week (MET-min/wk).

General autonomic function was quantified using the Composite Autonomic Symptom Score questionnaire (COMPASS-31). This questionnaire consists of 31 questions covering orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor domains of autonomic function (Sletten et al., 2012). Previous studies have utilized the COMPASS-31 to assess autonomic function in PwMS (Cortez et al., 2015; Sander et al., 2017).

Body Composition and Leg Fat-Free Mass

A whole-body Dual Energy X-Ray Absorptiometry (Hologic, Inc., Bedford, MA, USA) scan was performed on each participant to measure body composition. A region of interest was then drawn around the right quadricep, encompassing the entire upper leg with a diagonal line through the neck of the femur and terminating at the distal femur to quantify fat-free mass (FFM).

Peak Work Rate

Exercise capacity of the right quadricep was determined using a maximal work rate test, during which participants performed dynamic, graded knee extension exercise on a custom cycle ergometer at a rate of 40 revolutions per min. Participants were provided real-time feedback of their kicking rate using a cadence meter displayed on a screen directly in front of the ergometer. The test began with 2 min of unloaded, single-leg kicking followed by a 2.5 W increase in workload each min. The test was stopped when the participant could no longer maintain the target cadence, and the last complete stage was considered WRpeak.

Visit 2

Plasma Measures

Participants reported to the lab in the morning hours following an overnight fast. A catheter was then placed in an antecubital vein for serial blood sampling throughout the visit. Following a period of quiet rest, samples were acquired to assess basal levels of inflammation (IL-8 and IL-10) and oxidative stress (Protein Carbonyl). Additional blood samples were collected immediately prior to the onset of exercise and during the last 30 seconds of each exercise stage outlined below to measure plasma concentrations of norepinephrine and ET-1. Inflammation, oxidative stress, and ET-1 were assessed using standard methods of the Clinical and Translational Research Centers at the Children's Hospital Colorado, while norepinephrine was analyzed using validated methods at the Mayo Clinic (Rochester, MN).

Graded Knee-Extensor Exercise

Four exercise stages were performed on the cycle ergometer as described above. Two stages were completed at relative workloads of 20% WRpeak and 40% WRpeak, and two stages were completed at absolute workloads of 5 W and 10 W. The stages were completed in increasing order of intensity for each participant, and lasted 3-5 min.

Leg Blood Flow and Vascular Conductance

Common femoral artery mean blood velocity (MBV) and diameter were measured on the right leg with a 12 MHz linear array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA). Common femoral artery diameter was analyzed offline (Brachial Analyzer, Medical Imaging Applications, Coralville, IA, USA) using images that were acquired at end-diastole as determined by ECG gating (Vascular Imager, Medical Imaging Applications, Coralville, IA, USA) immediately following each stage with the leg stopped at a 90° angle. Blood velocity

measures were performed using a probe insonation angle < 60° and a frequency of 5MHz. MBV was calculated as a weighted mean of the spectrum of Doppler shift frequencies analyzed via a Multigon 500 M TCD spectral analyzer (Multigon Industries, Mount Vernon, NY, USA). Leg blood flow (LBF) and leg vascular conductance (LVC) were normalized to leg FFM and calculated using the equations:

$$LBF\ \left(\frac{mL}{min/Leg\ FFM(kg)}\right)$$

$$= MBV\ \left(\frac{cm}{s}\right) x\ \pi\ \left(\frac{Common\ Femoral\ Artery\ Diameter\ (cm)}{2}\right)^2 x \frac{60\ s}{min}\ x\ Leg\ FFM^{-1}$$

$$LVC\ \left(\frac{mL}{min/100\ mmHg/Leg\ FFM(kg)}\right)$$

$$= \frac{LBF\ \left(\frac{mL}{min}\right)}{Mean\ Arterial\ Pressure\ (mmHg)} x\ 100\ x\ Leg\ FFM^{-1}$$

Heart Rate and Blood Pressure

HR was calculated using a 3-lead electrocardiogram and mean arterial blood pressure (MAP) was non-invasively measured on a beat-by-beat basis using a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) (Chin and Panerai, 2012).

Hemodynamic Data Acquisition and Analysis

Data were collected at 250 Hz and analyzed offline using data acquisition and signal-processing software (WinDaq; DATAQ Instruments, Akon, OH, USA). Baseline and steady-state LBF, MAP, LVC, and HR were calculated over 30 s averages at rest and steady-state for each exercise intensity.

Statistical Analysis

Normality of participant characteristics, baseline hemodynamics, and baseline plasma markers was determined using the Shapiro Wilk test. Group differences were assessed using T-Tests and Wilcoxon Signed Rank Tests for parametric and non-parametric data, respectively. Cohen's D effect size was calculated for group comparisons of baseline hemodynamics and interpreted as small (d = 0.2), medium (d = 0.5) or large (d = 0.8) (J. Cohen, 1988). Exercise responses were analyzed using two-way repeated measures analysis of covariance (Group x Intensity) with baseline values included in the model as a covariate. Residual diagnostic plots were used to assess model assumptions, and participants with missing baseline values were excluded from the analysis. Plasma norepinephrine values during absolute exercise intensities were log transformed to satisfy model assumptions. Pairwise comparisons were made when a significant main effect or interaction was identified. In order to further interpret group differences, Eta-squared effect size (η^2) was calculated for the main effects and the Group x Intensity interaction. η^2 was interpreted as small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), or large ($\eta^2 = 0.14$) (J. Cohen, 1988). All data were analyzed using R (R Core Team, 2021) and SPSS (SPSS Inc., Chicago, IL) and significance was determined using an alpha of 0.05. One control participant was excluded from LBF and LVC analyses due to an inadequate Doppler signal, and plasma norepinephrine values during absolute intensities were identified as outliers and excluded in one participant.

RESULTS

Participant Characteristics

Participant characteristics are presented in Table 3.1. The two groups were matched for all characteristics. The MS cohort was mild-to-moderately disabled as indicated by PDDS scores ranging from 0-3 and a disease duration of approximately 14 years.

Table 3.1: Participant characteristics

	CTRL	*MS*
n	8 (7F, 1M)	8 (7F, 1M)
Age, y	55.4 ± 11.5	51.4 ± 11.0
Body Mass, kg	71.1 ± 9.9	63.3 ± 9.5
Height, cm	171.1 ± 9.0	165.8 ± 7.1
BMI, kg/m^2	24.2 ± 2.2	23.0 ± 3.3
Leg FFM, g	4882 ± 819	4892 ± 517
WRpeak, W	22.2 ± 4.5	18.1 ± 6.2
Physical Activity, MET-min/wk	1945 ± 1677	1230 ± 1152
COMPASS-31	8.7 ± 7.7	18.7 ± 11.7
Disability Status, PDDS	-	0-3
Disease Duration, y	-	13.6 ± 9.6

Values are mean ± SD. FFM, fat-free mass; WRpeak, peak work rate; W, watts; MET, metabolic equivalents; COMPASS-31, composite autonomic symptom score questionnaire; PDDS, patient determined disease steps questionnaire. $P > 0.05$ for all group comparisons.

Baseline Hemodynamics and Plasma Markers

Baseline hemodynamics are presented in Table 3.2. Although there were no significant differences, there was a medium effect size for increased baseline MAP and reduced LVC in the MS cohort. There were also no group differences in baseline plasma markers of norepinephrine, ET-1, inflammation, or oxidative stress (Table 3.3). However, there was a large effect size for reduced oxidative stress, as indicated by protein carbonyl concentration, in this group of PwMS (Table 3.3).

Table 3.2: Baseline hemodynamics

	CTRL	*MS*	*P-Value*	*Cohen's D*
HR, beats/min	59 ± 3	62 ± 9	0.38	-0.46
MAP, mmHg	93.0 ± 15.2	100.4 ± 9.1	0.26	-0.59
LBF, ml/min/kg FFM	42.6 ± 21.5	35.1 ± 14.4	0.45	0.42
LVC, ml/min/100 mmHg/ kg FFM	46.7 ± 21.5	35.9 ± 18.3	0.32	0.54

Values are mean ± SD. HR, heart rate; MAP, mean arterial pressure; LBF, leg blood flow; FFM, fat-free mass; LVC, leg vascular conductance

Table 3.3: Baseline plasma markers

	CTRL	*MS*	*P-Value*	*Cohen's D*
NE, pg/ml	897 ± 387	683 ± 387	0.41	0.44
ET-1, pg/ml	1.90 ± 0.38	1.98 ± 0.61	0.76	-0.16
Protein Carbonyl, nmol/mg	0.72 ± 0.09	0.62 ± 0.14	0.12	0.83
IL-8, pg/ml	4.87 ± 0.84	5.28 ± 2.05	0.62	-0.26
IL-10, pg/ml	0.34 ± 0.28	0.25 ± 0.14	0.85	0.41

Values are mean ± SD. NE and ET-1 sampled in the seated position prior to the start of exercise. Protein Carbonyl, IL-8, and IL-10 sampled in a supine position prior to the exercise task. NE, norepinephrine; ET-1, endothelin-1; IL-8, interleukin-8; IL-10, interleukin-10

Exercise Hemodynamics and Plasma Markers

As expected, HR, MAP, LBF, and LVC increased with intensity in both groups (Table 3.4). However, there were no group differences in the HR or MAP response to exercise (Figure 3.1; Tables 3.4 and 3.5). The PwMS had a significantly lower LBF response to exercise at 10 W relative to the control group (Figure 3.2A; Table 3.5). The plasma norepinephrine response to exercise at 10 W was greater in the MS group than the control group (Figure 3.3A, Table 3.5).

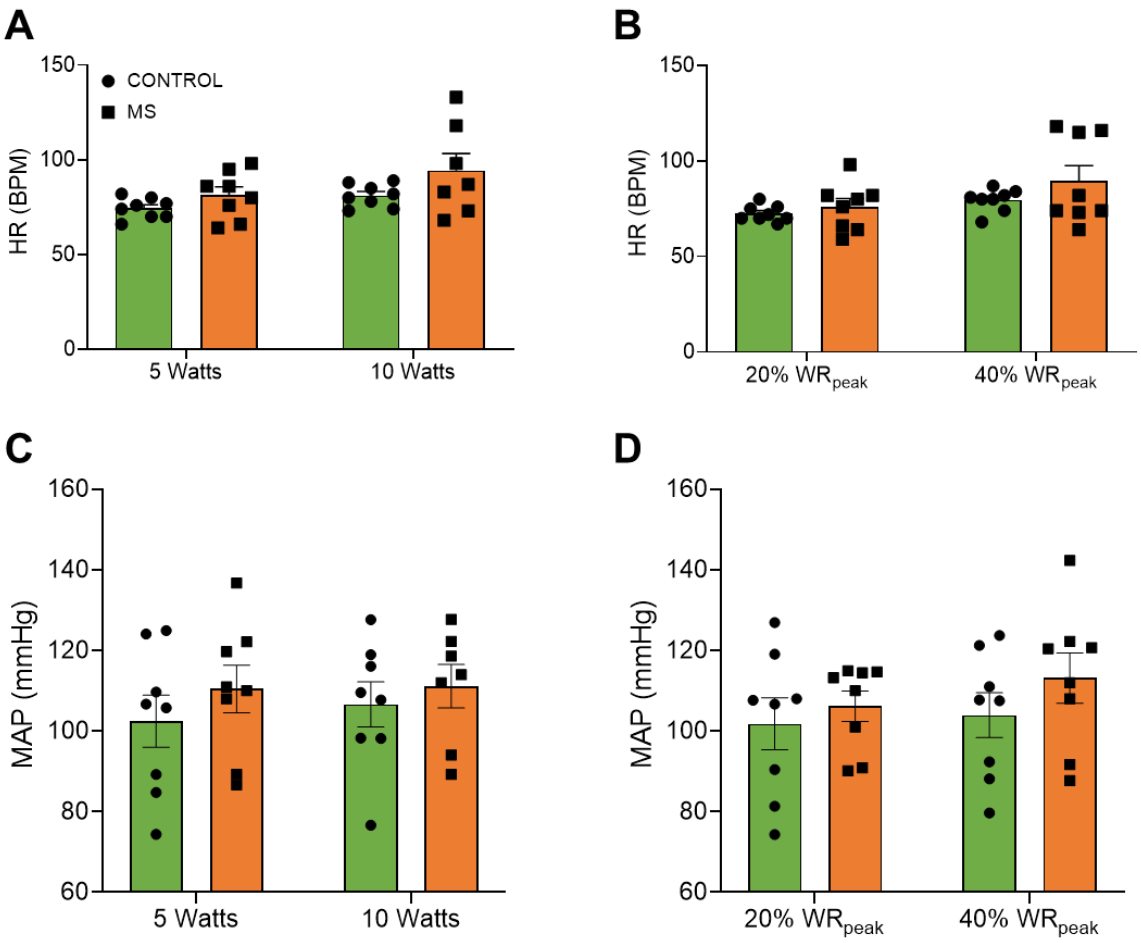

Figure 3.1: Heart rate and blood pressure responses to knee-extensor exercise

Group and individual data showing heart rate (A, B) and blood pressure (C, D) responses to knee-extensor exercise at absolute (A, C) and relative (B, D) workloads in healthy controls and participants with multiple sclerosis. HR, heart rate; MAP, mean arterial pressure; MS, multiple sclerosis; WRpeak, peak work rate. $P > 0.05$ for all group comparisons.

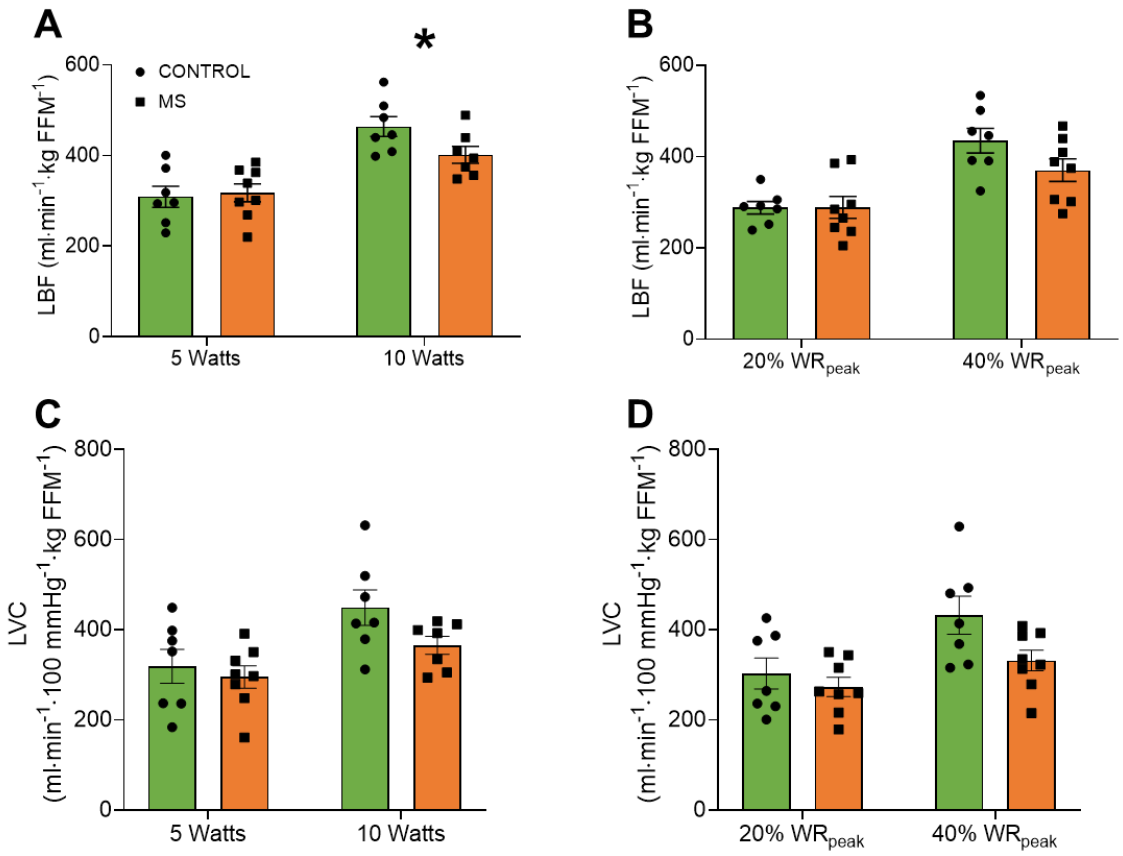

Figure 3.2: Blood flow and vascular conductance responses to knee-extensor exercise

Group and individual data showing blood flow (A, B) and vascular conductance (C, D) responses to knee-extensor exercise at absolute (A, C) and relative (B, D) workloads in healthy controls and participants with multiple sclerosis. LBF, leg blood flow; LVC, leg vascular conductance; MS, multiple sclerosis; WRpeak, peak work rate. * $P < 0.05$ between groups at same intensity

Table 3.4: Exercise response statistics

	Absolute Intensities		**Relative Intensities**	
	P-Value	*Eta-Squared*	*P-Value*	*Eta-Squared*
HR				
Group	0.22	0.14	0.57	0.08
Intensity	< 0.001	0.620	< 0.001	0.53
Group x Intensity	0.17	0.16	0.24	0.10
MAP				
Group	0.99	0.02	0.87	0.05
Intensity	0.02	0.40	0.02	0.33
Group x Intensity	0.91	< 0.01	0.19	0.12
LBF				
Group	0.38	0.05	0.29	0.10
Intensity	< 0.001	0.90	< 0.001	0.78
Group x Intensity	< 0.01	0.56	0.09	0.21
LVC				
Group	0.30	0.09	0.20	0.17
Intensity	< 0.001	0.78	< 0.001	0.71
Group x Intensity	0.02	0.40	0.05	0.26
Plasma NE				
Group	0.03	0.26	0.24	0.16
Intensity	0.28	0.12	0.29	0.09
Group x Intensity	0.20	0.16	0.29	0.09
Plasma ET-1				
Group	0.93	< 0.01	0.91	< 0.01
Intensity	0.32	0.07	< 0.01	0.53
Group x Intensity	0.66	0.02	0.73	0.13

HR, heart rate; MAP, mean arterial pressure; LBF, leg blood flow; LVC, leg vascular conductance; NE, norepinephrine; ET-1, endothelin-1

Table 3.5: Absolute values

	Control	MS	
	Mean ± SD	*Mean ± SD*	*P-Value*
HR			
5 Watts	74 ± 5	81 ± 12	0.46
10 Watts	81 ± 6	94 ± 24	0.11
20% WRpeak	73 ± 4	76 ± 13	0.96
40% WRpeak	80 ± 6	89 ± 22	0.29
MAP			
5 Watts	102.4 ± 18.2	110.4 ± 16.6	0.96
10 Watts	106.6 ± 15.7	111.1 ± 14.3	0.99
20% WRpeak	101.8 ± 18.2	106.2 ± 10.7	0.53
40% WRpeak	103.9 ± 15.8	113.1 ± 17.6	0.75
LBF			
5 Watts	309.0 ± 61.2	317.8 ± 56.3	0.64
10 Watts	464.1 ± 58.3	401.6 ± 49.7	0.05
20% WRpeak	291.5 ± 37.8	288.7 ± 68.2	0.95
40% WRpeak	434.8 ± 71.5	370.1 ± 69.7	0.08
LVC			
5 Watts	318.8 ± 99.5	294.9 ± 69.8	0.85
10 Watts	449.2 ± 103.9	365.2 ± 52.7	0.08
20% WRpeak	302.8 ± 90.4	273.0 ± 60.2	0.65
40% WRpeak	432.0 ± 111.5	331.5 ± 64.8	0.06
Plasma NE			
5 Watts	636.4 ± 156.4	691.4 ± 347.5	0.18
10 Watts	627.7 ± 140.1	639.2 ± 160.5	0.01
20% WRpeak	782.4 ± 305.1	681.7 ± 323.7	0.75
40% WRpeak	707.1 ± 223.2	681.7 ± 303.7	0.11
Plasma ET-1			
5 Watts	1.9 ± 0.4	1.9 ± 0.4	0.88
10 Watts	1.7 ± 0.3	1.8 ± 0.6	0.77
20% WRpeak	2.0 ± 0.4	2.0 ± 0.5	0.97
40% WRpeak	1.7 ± 0.4	1.7 ± 0.4	0.81

P-values are pairwise comparisons between groups at each intensity while controlling for baseline values of each measure. HR, heart rate; MAP, mean arterial pressure; LBF, leg blood flow; LVC, leg vascular conductance; NE, norepinephrine; ET-1, endothelin-1

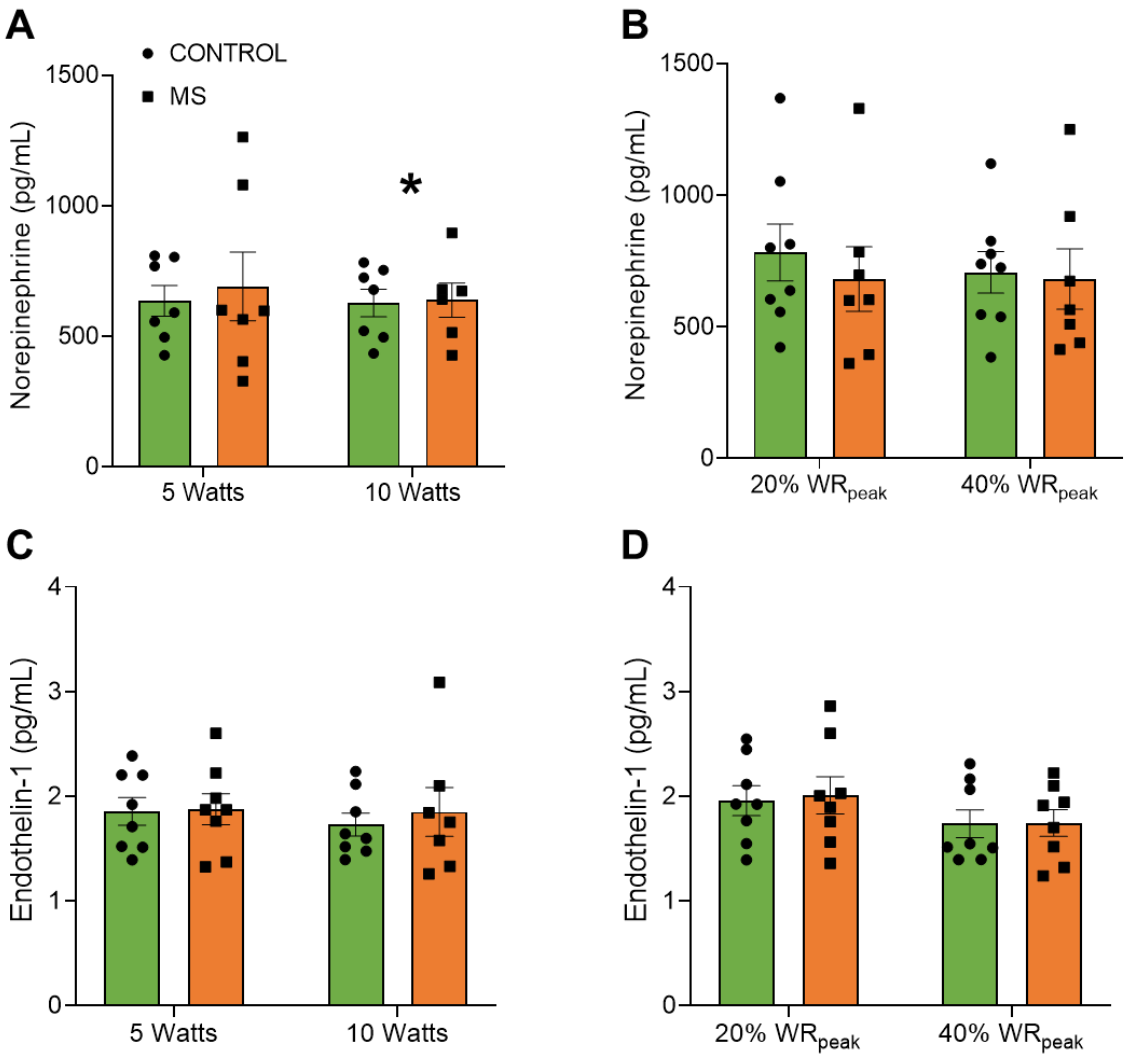

Figure 3.3: Plasma norepinephrine and endothelin-1 responses to knee-extensor exercise

Group and individual data showing plasma norepinephrine (A, B) and endothelin-1 (C, D) responses to knee-extensor exercise at absolute (A, C) and relative (B, D) workloads in healthy controls and participants with multiple sclerosis. MS, multiple sclerosis; WRpeak, peak work rate. * $P < 0.05$ between groups at same intensity.

DISCUSSION

The current study was designed on the premise that approximately 50% of PwMS experience cardiovascular autonomic dysfunction, and previous data demonstrating increased ET-1 mediated vascular tone in this clinical population. We hypothesized PwMS would have reduced skeletal muscle blood flow during exercise that engages the autonomic nervous system,

and that this reduction in blood flow would coincide with attenuated plasma norepinephrine and elevated ET-1. These data provide strong evidence in support of our hypothesis that skeletal muscle blood flow is reduced during exercise in PwMS. However, the plasma concentrations of norepinephrine and ET-1 do not agree with our original hypothesis, and further consideration of these findings is needed.

Baseline Hemodynamics and Plasma Markers

Although no baseline hemodynamic and plasma marker comparisons reached statistical significance, several findings warrant further discussion. We observed a medium effect size for increased MAP in the MS cohort, which contributed to a medium effect size for reduced LVC in these individuals (Table 3.2). The literature examining whether PwMS are at increased risk for hypertension is quite variable (Wens et al., 2013), and we cannot dismiss the possibility that we would have identified greater baseline MAP in the MS group if we studied more participants. Indeed, there is evidence to suggest that PwMS can experience adrenergic hyperactivity when presented with an orthostatic challenge (Habek, Pucić, et al., 2020). Moreover, the presence of white matter lesions and autonomic dysfunction is associated with supine hypertension (Milazzo et al., 2015). These findings highlight the importance of considering posture when evaluating blood pressure in MS; however, our baseline measures of plasma norepinephrine in this position indicate that SNA was not elevated in the PwMS. In fact, plasma norepinephrine was approximately 24% lower in the MS group prior to exercise, which is in agreement with previously published data at rest (Keller et al., 2014), but this did not reach significance due to the high variability associated with this measure.

MS is an inflammatory disease, and as a result, plasma markers of inflammation and oxidative stress are frequently elevated in PwMS (Ibitoye et al., 2016; Kallaur et al., 2017;

Oliveira et al., 2012; Patejdl et al., 2016). The detrimental impact of increased inflammation and oxidative stress on vascular function is well established (el Assar et al., 2013; Rodriguez-Manas et al., 2009), and we aimed to compare plasma concentrations of these markers between our groups. We found no evidence to indicate that the PwMS had increased levels of inflammatory markers, and actually identified a large effect size for lower oxidative stress as measured by plasma protein carbonyl in the MS group (Table 3.3). MS is a heterogenous disease and there is evidence to suggest that these compounds are not elevated in all individuals, particularly during periods of disease remission (Giovannoni et al., 2001; Soilu-Hänninen et al., 2005; Fjeldstad et al., 2011; Jankowska-Lech et al., 2015). Moreover, plasma concentrations of inflammation and oxidative stress biomarkers are positively associated with greater disability status (Oliveira et al., 2012; Kallaur et al., 2017), and the similar levels between groups may be due to the mild-to-moderate disability status of the PwMS. These data are in agreement with the interpretation of our previous findings during handgrip exercise, and the biomarkers measured in this study further indicate that inflammation and oxidative stress are unlikely to impact skeletal muscle blood flow during exercise in mild-to-moderately disabled PwMS.

Exercise Hemodynamics

Previous work demonstrating attenuated HR and blood pressure responses to dynamic exercise in PwMS was influential in the development of our hypothesis that skeletal muscle blood flow is reduced in PwMS during exercise (Cohen et al., 1989; Senaratne et al., 1984). However, the MS cohort in the current study had similar HR and MAP responses when compared to the control group (Figure 3.1, Tables 3.4 and 3.5). We expect that this is due to the mild-to-moderate disability status of our MS group relative to the previous studies. Despite these findings, we found strong evidence to support our hypothesis at the higher exercise

intensities of 10 W and 40% WRpeak. LBF was lower in the PwMS when exercising at 10 W, and nearly significant during the 40% WRpeak intensity (Figure 3.2, Table 3.5). The pairwise comparison of LVC at these intensities also provided strong evidence for a reduced dilatory response in the PwMS (Table 3.5). The lack of findings at the lower intensities and evidence of reduced LBF and LVC at the higher intensities in the MS group indicates that the magnitude of impairment in PwMS may increase with exercise intensity.

Plasma Measures During Exercise

In contrast to our hypothesis, plasma norepinephrine was greater in the PwMS during the absolute intensities when controlling for baseline values (Figure 3.3, Tables 3.4 and 3.5). Although not significantly lower, WRpeak in the MS group was 14% lower than the controls, and the absolute intensities may have required greater effort for the PwMS. Previous studies also indicate that ET-1 release increases during knee-extensor exercise (Wray et al., 2007; Barrett-O'Keefe et al., 2013), and this was of particular interest for the current study due to evidence to suggest that increased plasma ET-1 concentrations in PwMS restricts cerebral blood flow (D'Haeseleer et al., 2013). We did not identify group differences in ET-1 concentrations at any intensity (Figure 3.3, Tables 3.4 and 3.5), and key findings from a previous study demonstrating increased ET-1 in PwMS were obtained from an MS group with greater disability than the cohort in the current study (D'Haeseleer et al., 2013). It is important to note that if the discrepancy in findings between these studies is in fact due to differences in disability status, PwMS with greater disability scores may experience further decrements in skeletal muscle blood flow due to ET-1 mediated vasoconstriction.

Limitations and Future Directions

The purpose of this study was to determine whether PwMS have reduced skeletal muscle blood flow during exercise that engages the autonomic nervous system. Traditional, whole-body exercise places great demand on the sympathetic nervous system to increase cardiac output and constrict non-exercising vascular beds to ensure adequate perfusion of the active skeletal muscle. While the exercise modality used in this experiment is known to increase SNA (Notarius et al., 2019), the HR response to exercise in our participants clearly demonstrates that the magnitude of sympathetic engagement is less than that of whole-body exercise. However, the single-leg model was necessary to accurately measure skeletal muscle blood flow in a non-invasive manner.

Exercise elicits profound vasodilation in active skeletal muscle, and there are many factors that contribute to this response. Potassium released from contracting muscle, metabolic byproducts such as adenosine and CO_2, endothelium-mediated substances including nitric oxide and prostaglandins, and adenosine triphosphate released from erythrocytes following deoxygenation are just a few examples of the highly redundant dilatory factors that contribute to exercise hyperemia (Joyner and Casey, 2015). Our study focused on norepinephrine and ET-1 as mediators of vasoconstriction, as well as the possibility of reduced nitric oxide due to increased inflammation and oxidative stress in PwMS. Although our findings do not support a role for the influence of inflammation and oxidative stress, the potential influence of other dilatory signaling pathways warrants further investigation. However, our previous findings using the hand grip exercise model provide preliminary evidence against the hypothesis that altered local release of dilatory compounds impairs skeletal muscle blood flow during exercise in PwMS.

The findings of this study highlight the importance of autonomic activity and its impact on the regulation of skeletal muscle blood flow in PwMS. Approximately 50% of PwMS have

abnormal responses to tests of cardiovascular autonomic function (Huang, Jay and Davis, 2015), and it is important to determine whether PwMS suffering from cardiovascular autonomic dysfunction are at increased risk for compromised exercise hyperemia. Moreover, our plasma norepinephrine data indicate that PwMS may experience exaggerated α-adrenergic tone during exercise, but this hypothesis is based on data from other clinical populations. Future studies are needed to directly assess end organ responsiveness to sympathetic engagement in conjunction with indices of SNA to better understand α-adrenergic sensitivity in PwMS.

Perspectives and Conclusions

Skeletal muscle blood flow is directly associated with exercise capacity and plays an important role in fatigue during exercise (Hepple, 2002; Joyner & Casey, 2015; Kent et al., 2016). These data provide the first evidence to suggest that MS impairs the regulation of skeletal muscle blood flow during exercise. These findings open the door to a new field of research in MS to better understand exercise intolerance and fatigability in these individuals.

Previous data indicate that PwMS experience reduced SNA and plasma norepinephrine at rest (Keller et al., 2014), and we hypothesized that the MS group would have lower norepinephrine levels during exercise. However, plasma norepinephrine was actually greater in the PwMS when controlling for baseline levels, which were 24% lower in the MS group. These data provide evidence for a greater net increase in plasma norepinephrine in PwMS during exercise, which may exaggerate vascular tone. Indeed, clinical populations with chronically reduced SNA demonstrate hypersensitivity to α-adrenergic stimulation (Bannister et al., 1979; Biaggioni, Robertson and Robertson, 1994; Dejgaard et al., 1996). Together, our findings indicate that PwMS may experience greater α-adrenergic tone during exercise, which ultimately limits skeletal muscle blood flow and dilatory capacity in the active tissue.

This study provides evidence in support of the hypothesis that PwMS experience reduced skeletal muscle blood flow during exercise that engages the autonomic nervous system. Although future studies are needed to better understand the mechanisms leading to compromised perfusion of active skeletal muscle, the current data suggests that α-adrenergic constriction via greater release of norepinephrine may play a role.

CHAPTER 4 – MANUSCRIPT 3

Metaboreflex stimulation of sympathetic nerve activity in persons with multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, degenerative disease of the central nervous system that causes a wide array of symptoms including autonomic nervous system dysfunction. Previous work utilizing direct measures of muscle sympathetic nerve activity (SNA) and plasma concentrations of norepinephrine demonstrate that persons with MS (PwMS) have reduced SNA at rest relative to healthy individuals (Keller et al., 2014). While the consequences of reduced basal SNA are not fully understood in PwMS, data from other clinical populations with chronically reduced SNA suggest that PwMS may actually experience hypersensitivity to α-adrenergic stimulation (Bannister et al., 1979; Biaggioni, Robertson and Robertson, 1994; Dejgaard et al., 1996). Indeed, previous findings from our lab suggest that increased α-adrenergic mediated vasoconstriction may contribute to reduced skeletal muscle blood flow during exercise in PwMS. Thus, it is important to gain a better understanding of sympathetic control of the vasculature in this clinical population.

The metaboreflex test offers a controlled manner in which to assess engagement of, and responsiveness to SNA. The test traditionally consists of a challenging exercise task, followed by a period of post-exercise muscle ischemia achieved via occlusion of the active tissue to trap metabolites and increase SNA via activation of metabolically sensitive group III / IV afferents. Previous data utilizing the metaboreflex test in PwMS offer key insights to altered sympathetic control of the vasculature in these individuals. Although PwMS were able to increase blood pressure in response to post-exercise muscle ischemia similarly to controls, they achieved this

response via increased systemic vascular resistance as opposed to increased cardiac output (Q) (Marongiu et al., 2015). Importantly, this response is also observed in individuals with cardiovascular and metabolic disease (Crisafulli et al., 2007; Choi et al., 2013; Milia et al., 2015). Moreover, a follow-up study by this group demonstrated that the altered response to muscle metaboreflex activity in PwMS is not due to physical deconditioning and is instead likely a consequence of the disease (Magnani et al., 2016).

Together, these findings support the hypothesis that PwMS may experience hypersensitivity to α-adrenergic stimulation, which manifests as exaggerated vasoconstriction. However, previous studies have not assessed engagement of the sympathetic nervous system in combination with the systemic response to the metaboreflex test. As a result, it is unknown whether altered responsiveness to the metaboreflex test in PwMS is due to differing SNA and plasma norepinephrine, end-organ responsiveness to the SNA, or a combination of both. Therefore, the purpose of this study was to simultaneously assess SNA, as indicated by plasma norepinephrine concentrations, and systemic responses to the metaboreflex test in PwMS and healthy controls.

METHODS

The metaboreflex protocol was performed following 45 min of quiet rest in the supine position after the conclusion of the experiments performed in Chapter 3. Eight healthy control participants (7F, 1M) and 8 PwMS (7F, 1M) completed the testing. All participants were non-smokers, sedentary to moderately physically active, and not taking medications to control blood pressure or cholesterol. Participants were required to not have been hospitalized or had any changes to their medications within 3 months of starting the study, and all participants in the MS cohort were free of relapses for at least 3 months prior to the study. Disability status of the MS cohort and physical activity levels of all participants were determined using the methods

described in Chapter 3. All experiments were performed in a temperature-controlled laboratory (20 - 22°C), and the procedures were approved by the Colorado State University Institutional Review Board and were conducted in accordance with the Declaration of Helsinki.

Maximal Hand Grip Strength

Maximal hand grip strength was measured on the opposite arm of the venous catheter using an electronic hand grip dynamometer (Stoelting Co., Wood Dale, IL, USA). A series of 3-5 maximal voluntary contractions (MVC) were performed with strong verbal encouragement by the study team until an increase in force was no longer observed in consecutive contractions. The greatest force output was documented as the MVC and used to determine target force output during the metaboreflex test.

Metaboreflex Protocol

The metaboreflex protocol was performed with all participants in a supine position and the exercising hand resting comfortably at their side. Following acquisition of baseline data, participants rapidly increased grip force to 30% of their MVC. The force output of the sustained isometric contraction was displayed in real time on a computer screen for the participants. Participants were instructed to maintain their target force output for as long as possible while the research team provided verbal encouragement and reminded the participants to continue breathing to avoid the Valsalva maneuver. A cuff was rapidly inflated around the exercising forearm to approximately 220 mmHg (Hokanson, Bellevue, WA) immediately before the participant was instructed to relax due to an inability to maintain the target force output. Two min of post-exercise muscle ischemia was performed and all participants were instructed to relax and maintain a regular breathing pattern.

Plasma Measures

Plasma norepinephrine and endothelin-1(ET-1) were measured during supine rest and during the last 30 s of post-exercise muscle ischemia. Analyses were performed as described in Chapter 3.

Heart Rate, Blood Pressure, Cardiac Output, and Systemic Vascular Resistance

Heart rate (HR) was calculated using a 3-lead electrocardiogram, while mean arterial blood pressure (MAP) and Q were non-invasively measured on a beat-by-beat basis using a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) (Chin and Panerai, 2012). Systemic vascular resistance (SVR) was calculated as the quotient of MAP and Q.

Hemodynamic Data Acquisition and Analysis

Data were collected at 250 Hz and analyzed offline using data acquisition and signal-processing software (WinDaq; DATAQ Instruments, Akon, OH, USA). Baseline and post-exercise muscle ischemia values for HR, MAP, Q, and SVR represent the average over the last 30 s of each stage.

Statistical Analysis

Normality of participant characteristics was confirmed using the Shapiro Wilk test and group differences were assessed using T-Tests. Cohen's D effect size was calculated for group comparisons of baseline hemodynamics and interpreted as small (d = 0.2), medium (d = 0.5) or large (d = 0.8) (J. Cohen, 1988). Hemodynamics and plasma measures were analyzed using a two-way repeated measures analysis of variance (Group x Stage). The factor of stage had two levels – baseline and post-exercise muscle ischemia. Residual diagnostic plots were used to assess model assumptions. Some evidence of unequal variance was observed in diagnostic plots for Q, plasma norepinephrine, and plasma ET-1 data. Log and square root transformations were tried but did not noticeably improve assumptions for ET-1 data. Hence analysis was done on the

original scale for ET-1. Q and plasma norepinephrine data were log transformed to satisfy model assumptions. Eta-squared effect size (η^2) was calculated for the main effects and the Group x Stage interaction. η^2 was interpreted as small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), or large ($\eta^2 = 0.14$) (J. Cohen, 1988). Pairwise comparisons were performed when a significant main effect or interaction was identified. All data were analyzed using R (R Core Team, 2021) and SPSS (SPSS Inc., Chicago, IL) and significance was determined using an alpha of 0.05.

RESULTS

Participant Characteristics

Participant characteristics are presented in Table 4.1. There were no differences between groups in any of the measures of participant characteristics. The MS cohort was mild-to-moderately disabled as indicated by PDDS scores ranging from 0-3 and a disease duration of approximately 14 years.

Table 4.1 Participant characteristics

	CTRL	*MS*
n	8 (7F, 1M)	8 (7F, 1M)
Age, y	55.4 ± 11.5	51.4 ± 11.0
Body Mass, kg	71.1 ± 9.9	63.3 ± 9.5
Height, cm	171.1 ± 9.0	165.8 ± 7.1
BMI, kg/m^2	24.2 ± 2.2	23.0 ± 3.3
Non-Dominant MVC, kg	29.5 ± 9.9	25.8 ± 4.7
Physical Activity, MET-min/wk	1945 ± 1677	1230 ± 1152
COMPASS-31	8.7 ± 7.7	18.7 ± 11.7
Disability Status, PDDS	-	0-3
Disease Duration, y	-	13.6 ± 9.6

Values are mean ± SD. BMI, body mass index; MVC, maximal voluntary contraction; MET, metabolic equivalents; PDDS, patient determined disease steps questionnaire. *P* > 0.05 for all group comparisons.

Systemic Responses to Post-Exercise Muscle Ischemia

Both groups had similar HR and blood pressure responses to the metaboreflex stimulus; however, the control group increased Q relative to baseline, while the MS group did not (Figure 4.1, Tables 4.2 and 4.3). Although not statistically significant, there was a medium effect size for a greater change in SVR in the MS group (Figure 4.2B, $P = 0.20$, d = -0.75). There was a large effect size for the main effect of group, indicating potentially lower plasma norepinephrine in the PwMS (Table 4.2). However, the plasma norepinephrine response to post-exercise muscle ischemia was similar between the groups (Figure 4.3A, Tables 4.2 and 4.3). There were no group differences in ET-1 (Figure 4.3, Tables 4.2 and 4.3).

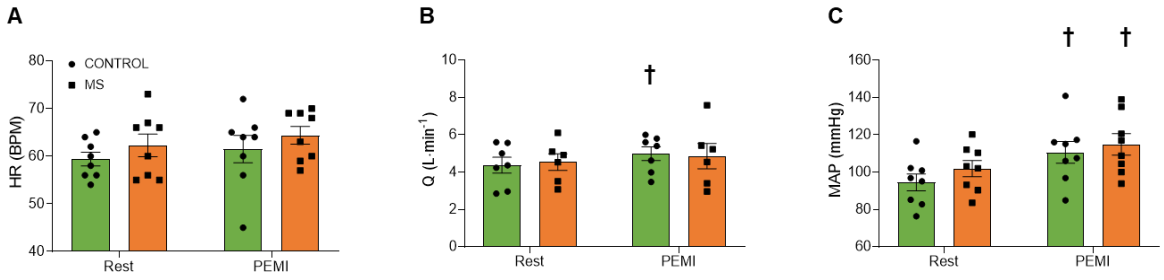

Figure 4.1: Heart rate, cardiac output, and blood pressure responses to post-exercise muscle ischemia

Group and individual data showing heart rate (A), cardiac output (B), and mean arterial pressure (C) at rest and the last 30 s of post-exercise muscle ischemia in healthy controls and participants with multiple sclerosis. MS, multiple sclerosis; HR, heart rate; Q, cardiac output; MAP, mean arterial pressure; PEMI, post-exercise muscle ischemia. † $P < 0.05$ vs. Rest within group.

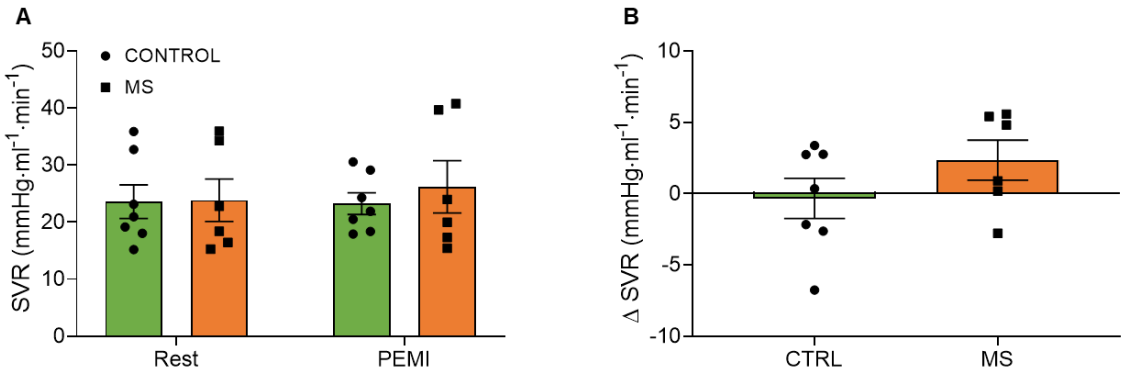

Figure 4.2: Systemic vascular resistance response to post-exercise muscle ischemia

Group and individual data showing systemic vascular resistance at rest and the last 30 s of post-exercise muscle ischemia (A) and the change in systemic vascular resistance in response to post-exercise muscle ischemia (B) in healthy controls and participants with multiple sclerosis. MS, multiple sclerosis; SVR, systemic vascular resistance; PEMI, post-exercise muscle ischemia. $P > 0.05$ for all group comparisons.

Table 4.2: Metaboreflex response statistics

	P-Value	*Eta-Squared*
HR		
Group	0.32	0.07
Intensity	0.13	0.15
Group x Intensity	1.00	< 0.01
Q		
Group	0.97	< 0.01
Intensity	0.01	0.46
Group x Intensity	0.14	0.18
MAP		
Group	0.42	0.05
Intensity	< 0.001	0.78
Group x Intensity	0.47	0.04
SVR		
Group	0.73	0.01
Intensity	0.34	0.08
Group x Intensity	0.21	0.14
Plasma NE		
Group	0.08	0.175
Intensity	0.01	0.38
Group x Intensity	0.97	< 0.01
Plasma ET-1		
Group	0.97	0.01
Intensity	0.11	0.15
Group x Intensity	0.79	0.03

HR, heart rate; Q, cardiac output; MAP, mean arterial pressure; SVR, systemic vascular resistance; NE, norepinephrine; ET-1, endothelin-1

Table 4.3: Absolute values

	Control	**MS**	
	Mean ± SD	*Mean ± SD*	*P-Value*
HR			
Rest	59 ± 4	62 ± 7	0.37
PEMI	62 ± 8	64 ± 5	0.37
P-Value	0.28	0.28	
Q			
Rest	4.4 ± 1.1	4.5 ± 1.1	0.77
PEMI	5.0 ± 1.0	4.9 ± 1.7	0.72
P-Value	< 0.01	0.34	
MAP			
Rest	94.5 ± 12.8	101.8 ± 12.3	0.33
PEMI	110.5 ± 16.5	114.8 ± 16.2	0.57
P-Value	< 0.001	< 0.001	
SVR			
Rest	23.6 ± 7.8	23.8 ± 9.1	0.96
PEMI	23.2 ± 5.0	26.2 ± 11.2	0.54
P-Value	0.81	0.14	
Plasma NE			
Rest	580.3 ± 243.8	433.8 ± 288.3	0.11
PEMI	780.9 ± 178.2	594.6 ± 311.0	0.12
P-Value	0.05	0.06	
Plasma ET-1			
Rest	1.7 ± 0.2	1.5 ± 0.3	0.90
PEMI	1.7 ± 0.5	1.5 ± 0.4	0.86
P-Value	0.17	0.33	

HR, heart rate; Q, cardiac output; MAP, mean arterial pressure; SVR, systemic vascular resistance; NE, norepinephrine; ET-1, endothelin-1; PEMI, post-exercise muscle ischemia

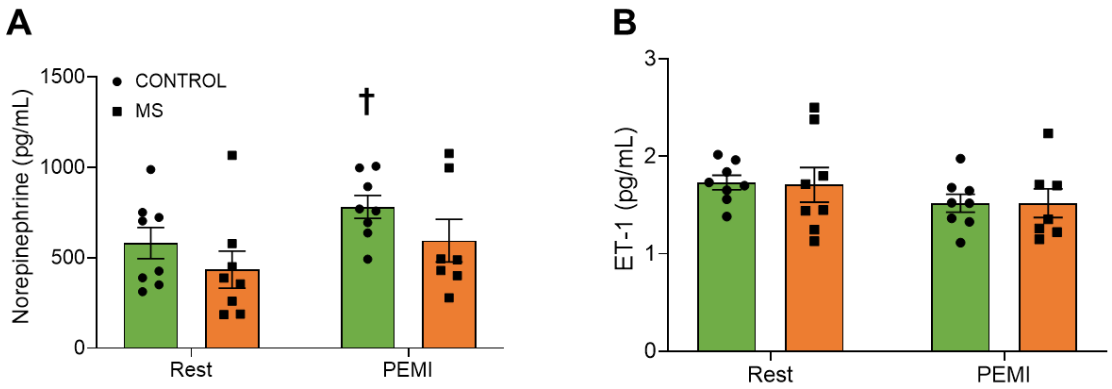

Figure 4.3: Plasma norepinephrine and endothelin-1 responses to post-exercise muscle ischemia

Group and individual data showing plasma norepinephrine (A) and endothelin-1 (B) at rest and the last 30 s of post-exercise muscle ischemia in healthy controls and participants with multiple sclerosis. MS, multiple sclerosis; ET-1, endothelin-1; PEMI, post-exercise muscle ischemia. † $P < 0.05$ vs. Rest within group.

DISCUSSION

This study was designed to measure engagement of, and responsiveness to SNA in PwMS. In agreement with previously published data, we found that PwMS failed to increase Q in response to metaboreflex stimulation via post-exercise muscle ischemia. Our data are also in alignment with previous findings demonstrating increased systemic vascular resistance during this stimulation. Moreover, our plasma norepinephrine data indicate for the first time that these responses likely occur at lower levels of sympathetic engagement in PwMS.

Previous studies investigating the response to metaboreflex stimulation demonstrate that PwMS have normal HR and blood pressure responses to the stimulus. As a result, the authors concluded that sympathetic responses are preserved in PwMS despite a failure to increase Q (Marongiu et al., 2015). Our data are in agreement with these findings; however, the plasma norepinephrine values suggest that while the magnitude of increase in SNA is similar between PwMS and controls, the MS group likely experienced these responses at lower absolute levels of SNA (Figure 4.3, Tables 4.2 and 4.3). It is important to note that previously published findings

on the metaboreflex in PwMS utilized a mild stimulation that, on average, increased mean arterial pressure by less than 10 mmHg (Marongiu et al., 2015; Magnani et al., 2016). Our study utilized a stronger stimulus in an effort to better represent the change in blood pressure seen during whole-body exercise, which resulted in a greater pressor response and likely required greater engagement of the sympathetic nervous system.

The metaboreflex test is used to measure the response to increased SNA. However, the plasma norepinephrine response observed during post-exercise muscle ischemia differed from previous findings in our lab during kicking exercise in PwMS. We previously found that PwMS have a greater increase in plasma norepinephrine during single-leg kicking than healthy controls. Alternatively, the plasma norepinephrine response during the metaboreflex test indicates that PwMS increase SNA similarly to controls, but that the absolute level of sympathetic engagement is likely lower. While these data do provide evidence of hypersensitivity to α-adrenergic stimulation in PwMS, they also highlight the importance of considering the stimulus when interpreting the cardiovascular response in this clinical population.

SNA is increased during dynamic exercise via a complex integration of signaling pathways including feed-forward activity from the motor cortex, as well as metabo- and mechano- reflex activity (Ludbrook and Graham, 1985; Mitchell, 1985; Rowell and O'Leary, 1990). By design, the metaboreflex test removes many of these factors and isolates the stimulation of SNA to the metabolic stimulation of chemoreceptors in the muscle. Thus, the differing net integration of afferent signaling pathways to increase SNA between these stimuli may explain the discrepancies in the plasma norepinephrine response between dynamic exercise and the metaboreflex test in PwMS.

It is hypothesized that an inability to increase Q during the metaboreflex leads to an exaggerated increase in systemic vascular resistance in order to maintain blood pressure. Cardiac dysfunction is common in MS, and parameters such as ejection fraction and stroke volume are compromised and likely contribute to reduced Q in these individuals (Olindo et al., 2002; Akgul et al., 2006; Wens, Eijnde and Hansen, 2016). It is important to note that although Fingolimod is known to impair ventricular function in MS (Racca et al., 2016), participants with any history of taking Fingolimod were excluded from the current study. While the potential effect of other drugs used in the MS group cannot be excluded, the net response is representative of what PwMS experience as a result of their disease and necessary medications.

Limitations and Future Directions

The current study was designed to better understand the cardiovascular response to increased SNA in PwMS. While the plasma measures of norepinephrine included in this study provided the first insight to sympathetic engagement and subsequent end-organ responsiveness in PwMS, these plasma measures are only an index of SNA. Direct measures of SNA using microneurography are ultimately needed to determine whether MS alters the response to sympathetic engagement. Moreover, intra-arterial infusions of sympathomimetics with corresponding measures of vascular tone would provide direct measures of sensitivity to SNA in PwMS.

The current results in combination with our previous data during dynamic exercise also reveal that the sympathetic response is likely specific to the stimulus. Moreover, data in healthy individuals demonstrate differing responses to stimuli that engage the sympathetic nervous system. For example, healthy controls increase blood pressure in response to post-exercise muscle ischemia via increased Q and little to no change in SVR (Figures 4.1 and 4.2, Table 4.3) (Marongiu *et al.*, 2015), whereas healthy individuals maintain blood pressure in response to an

orthostatic challenge via increased SVR as opposed to increased Q (Rowell, 1993). As a result, the findings in the current study may not translate to exercise or other challenges experienced by those living with MS, such as orthostatic intolerance.

Perspectives and Conclusions

PwMS are known to experience high levels of fatigue and reduced exercise capacity, but the underlying mechanisms of these symptoms are still poorly understood (de Haan et al., 2000; Langeskov-Christensen et al., 2015; Rudroff, Kindred and Ketelhut, 2016; Zijdewind, Prak and Wolkorte, 2016). Peripheral afferent signaling, such as the chemoreflex elicited during post-exercise muscle ischemia, can directly inhibit central command (Amann et al., 2008). Thus, altered metaboreflex activity has the potential to directly affect neuromuscular drive to the muscle, resulting in reduced force output and increased indices of fatigability. While the current and previous studies did not directly assess afferent neural signaling during post-exercise muscle ischemia, similar HR and blood pressure responses in the MS groups of each study suggest that MS likely does not affect the magnitude of afferent signaling. However, consistent evidence of increased systemic vascular resistance in response to sympathetic engagement during post-exercise muscle ischemia in PwMS indicates that these individuals may experience an exaggerated vasoconstrictor response. Indeed, the plasma norepinephrine concentrations at rest in this study are in agreement with previous data demonstrating reduced basal SNA in MS (Keller et al., 2014), and evidence from other clinical populations demonstrates that chronically reduced basal levels of SNA can lead to hypersensitivity to α-adrenergic signaling (Bannister et al., 1979; Biaggioni, Robertson and Robertson, 1994; Dejgaard et al., 1996). Together, these findings suggest that PwMS experience greater systemic vascular resistance and reduced skeletal muscle perfusion due to exaggerated α-adrenergic vasoconstriction. This reduction in skeletal

muscle perfusion would limit oxygen delivery and ultimately increase fatigue of the active muscle.

These data demonstrate that PwMS fail to increase Q in response to post-exercise muscle ischemia, and instead likely rely on augmented systemic vascular resistance to maintain blood pressure. Corresponding measures of plasma norepinephrine indicate that PwMS achieve this increase in systemic vascular resistance at lower absolute levels of SNA. Future studies are needed to directly assess sensitivity to α-adrenergic stimulation to better understand sympathetic control of the vasculature in MS.

CHAPTER 5 – PERSPECTIVES AND CONCLUSIONS

This series of studies provides important insight into the detrimental effects of multiple sclerosis (MS) on systemic and vascular control of skeletal muscle blood flow. In Experiment 1, we demonstrated that local control of skeletal muscle blood flow during rhythmic handgrip exercise in mild-to-moderately disabled persons with MS (PwMS) is intact. These findings indicate that basal levels of circulating vasoactive compounds, as well as those released during dynamic exercise, do not negatively affect skeletal muscle blood flow during exercise in PwMS. In Experiment 2, we showed for the first time that skeletal muscle blood flow during dynamic exercise that engages the autonomic nervous system is likely impaired in PwMS. Additionally, this experiment provided evidence to suggest that the release of norepinephrine is greater in PwMS during exercise. These findings provide the first evidence of compromised exercise hyperemia in PwMS and lay the foundation for a new line of research to better understand the underlying causes of reduced exercise capacity and increased fatigability in PwMS. Experiment 3 was designed to better understand engagement of, and response to, sympathetic nerve activity (SNA) in PwMS. Our findings were in agreement with previous studies utilizing the metaboreflex test in PwMS in that we found evidence of an inability to increase cardiac output (Q) and exaggerated systemic vascular resistance in PwMS. Furthermore, our measures of plasma norepinephrine provided the first index of SNA during the metaboreflex test in PwMS, and the findings suggest that MS is associated with a lower level of SNA during this test. Together, the findings from this series of experiments and previously published findings in MS and other clinical populations have led to our working hypothesis that skeletal muscle blood flow is impaired during exercise in PwMS due to exaggerated α-adrenergic vasoconstrictor tone as a

result of hypersensitivity to this signaling and an increased release of norepinephrine.

Future work is needed to better understand sympathetic control of the vasculature in PwMS. Recent developments in microneurography now allow for successful measurements of muscle SNA during cycle exercise (Moralez et al., 2018; Hansen et al., 2020), and experiments utilizing these techniques will help determine whether PwMS experience altered SNA during exercise. Additionally, direct measures of sensitivity to sympathetic signaling via intra-arterial infusions of sympathomimetics including phenylephrine (α_1-agonist) and dexmedetomidine (α_2-agonsit) will determine whether chronically reduced basal SNA in MS results in hypersensitivity to α-adrenergic signaling in. Moreover, the influence of endothelin-1 (ET-1) on vascular tone during exercise warrants further investigation. Although our findings indicate that plasma concentrations are not different in MS, ET-1 is released from the vascular endothelium of active tissue (Barrett-O'Keefe et al., 2013), and the non-invasive nature of our study prevented us from performing these calculations. Furthermore, our samples were taken from an antecubital vein and may not fully represent concentrations in the exercising tissue. Future studies comparing skeletal muscle blood flow responses with and without the administration of ET-1 antagonists are needed to ultimately determine whether ET-1 limits peripheral blood flow in a similar manner to what has been observed centrally in these individuals (D'Haeseleer et al., 2013).

Collectively, the results of these experiments reveal an important new line of questioning in our understanding of exercise capacity, fatigability, and sympathetic control in MS.

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