Abstract

The arterial baroreflex's (ABR) operating point (OP) pressure is reset upwards and rightwards from rest in direct relation to the increases in dynamic exercise intensity. However the interneural pathways and signaling mechanisms that lead to upwards and rightwards resetting of the OP pressure, and hence the increases in central sympathetic outflow during exercise, remain to be identified. Data from recent animal investigations have implicated nitric oxide (NO•) as a modulator of central sympathetic outflow. For example, introduction of NO• centrally dampens sympathetic outflow and there is a growing body of evidence that indicates that central NO• is scavenged by centrally generated free radicals (FR), thereby, enabling increased central sympathetic outflow. Furthermore, during dynamic exercise, increases in centrally generated FRs formed by increased intensity-related oxidative metabolism and central angiotensin II (Ang II) production linked to exercise intensity related FR production suggests that FRs are candidate signaling molecules. Whether the primary site of the FRs signaling action occurs within the central nervous system (CNS) or is a result of peripheral chemo- or mechano-receptor input to the CNS remains to be established. Therefore, the aim of the proposed research is to investigate the role of FRs on arterial baroreflex resetting in human subjects.

The first investigation of this project tested the hypothesis that combined central and peripheral FRs play a pivotal role in the exercise related resetting of arterial baroreflex control of arterial blood pressure and muscle sympathetic nerve activity (MSNA) in healthy subjects. The second investigation of this project tested the hypothesis that the Ang II linked FR production-mediated acute ABR-OP pressure resetting during exercise is located within the CNS. From these investigations we identified that: i) free radical production, particularly superoxide, plays a pivotal role in the exercise related rightward and upward resetting of the ABR-OP pressure and

the reflex control of central sympathetic outflow; and ii) the major effect of Ang II on ABR-OP pressure resetting and control of central sympathetic outflow occurs centrally.

THE ROLE OF FREE RADICALS IN THE EXERCISE INDUCED RESETTING OF THE ARTERIAL BAROREFLEX

Gilberto Moralez Jr., B.S, M.S.

APPROVED BY SUPERVISING COMMITTEE:

_____

Peter B. Raven, Ph.D., Major Professor

_____

Robert T. Mallet, Ph.D., Committee Member

_____

Tom Cunningham, Ph.D., Committee Member

_____

Nathalie Sumien, Ph.D., Committee Member

_____

Caroline A. Rickards, Ph.D., Committee Member

_____

Maya Nair, Ph.D., University Member

_____

Steven Mifflin, Ph.D., Department Chair

_____

Meharvan Singh, Ph.D., Dean, Graduate School of Biomedical Sciences

DEDICATION

This dissertation is dedicated in memory of my best friend growing up, my cousin Manuel Morales III (1983-2013) , my first dancing partner, my grandmother Bernadina Lozano (1946-2015) and my son Samuel Moralez (May 19, 2015 – May 23, 2015).

THE ROLE OF FREE RADICALS IN THE EXERCISE INDUCED RESETTING OF THE ARTERIAL BAROREFLEX

DISSERTATION

Presented to the Graduate Council of the

University of North Texas Health Science Center

At Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Gilberto Moralez Jr., BS, MS

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Original Articles

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Jouett, N. P., **Moralez, G**., White, D. W., Eubank, W. L., Chen, S., Tian, J., Smith, M. L., Zimmerman, M. C. and Raven, P. B. N-Acetylcysteine reduces hyperacute intermittent hypoxia-induced sympathoexcitation in human subjects. Exp Physiol, 101: 387–396, 2016

Moralez G, Romero SA, Rickards, CA, Ryan KL, Convertino VA, and Cooke WH. Effects of dehydration on cerebrovascular control during standing after heavy resistance exercise. *Journal of Applied Physiology*.112:1875-1889, 2012

Cooke WH., **Moralez G**, Barrera C, and Cox P. Digital infrared thermographic imaging for remote assessment of traumatic injury. *Journal of Applied Physiology*. 111:1813-1818, 2011.

Romero SA, **Moralez G**, Rickards CA, Ryan KL, Fogt DL, Convertino VA, and Cooke WH. Control of cerebral blood velocity with furosemide-induced hypovolemia and upright tilt. *Journal of Applied Physiology*. 110:492-498, 2011.

Published Abstracts

Moralez G, Jouett N, White DW, Zimmerman MC, Tian J, Chen S, Smith ML and Raven PB. Antioxidants Attenuate the Exercise Induced Increases in Muscle Sympathetic Nerve Activity during Heavy Dynamic Exercise. American College of Sports Medicine meeting, 2016 Boston, Massachusetts

Moralez G, Jouett N, Tian J, Zimmerman MC and Raven PB. Centrally Acting Perindopril Attenuates the Exercise Induced Increase in Muscle Sympathetic Nerve Activity during Heavy Dynamic Exercise. Experimental Biology meeting 2016, San Diego, California

LeBlanc A, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, Smith SM, Evans H, Spector E, Snyder RP, Sibonga J, Keyak J, Nakamura T, Kohri K, Ohshima H, **Moralez G.** Spaceflight Bone Atrophy-Problem Solved? American Society for Bone and Mineral Research Meeting. Seattle, Washington, October 2015.

Jouett N, **Moralez G**, Raven PB and Smith ML. N-Acetyl Cysteine Attenuates Intermittent Hypoxia Induced Sympathoexcitation in Human Subjects. FASEB J April 2015 29:955.4

LeBlanc A, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, Smith SM, Evans H, Spector E, Ploutz-Snyder R, Sibonga J, Keyak J, Nakamura T, Kohri K, Ohshima H, **Moralez G.** Update of the Bisphosphonate ISS Flight Experiment. NASA Human Research Program Investigators' Workshop (HRP IWS 2015), Galveston, TX, January,2015 JSC-CN-32238

LeBlanc A, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, Smith SM, Evans H, Spector E, Ploutz-Snyder R, Sibonga J, Keyak J, Nakamura T, Kohri K, Ohshima H, **Moralez G.**

Update of the Bisphosphonate ISS Flight Experiment. American Society for Bone and Mineral Research; Sept. 2014; Houston, TX, Galveston, TX, JSC-CN-30935

Moralez G, White DW, and Raven PB. Effects of antioxidants on cerebrovascular control during moderate and high intensity exercise. *FASEB J* April 2014 28:1183.6

White DW, Kay VL, **Moralez G**, Benson T, Carlton J, Eubank W and Raven PB. Effect of posture on heart rate control prior to and following dynamic leg exercise. *FASEB J* April 2014 28:881.8

Moralez G, Romero SA, Ryan KL, Cooke WH, Rickards CA. Effects of inspiratory resistance on cerebral blood velocity during orthostasis with dehydration. *FASEB J* 2013; 27:1203.14.

Eubank W, White DW, **Moralez G**, Kay V, Watenpaugh DE and Raven PB. Role of Reactive Oxygen Species in Intermittent Hypoxia Induced Sympathoexcitation. *FASEB J* April 9, 2013 27:938.2.

White DW, Kay V, **Moralez G**, Eubank W and Raven PB. A Case for a Standardized Method for the Normalization of Muscle Sympathetic Nerve Activity (MSNA) Amplitude. FASEB J April 9, 2013 27:1117.1

Moralez G, Romero SA, Rickards CA, Raven PB, and Cooke WH. Cerebrovascular Hemodynamics during Concentric and Eccentric Phases of Heavy Resistance Exercise. *International Journal of Exercise Science*. 2, Article 14, 2013.

Moralez G, Cox P, Lucas RAI, Ganio MS, Pearson J, Crandall CG, and Cooke WH. Infrared thermal imaging of human skin temperature during combined simulated hemorrhage and thermal stress. FASEB J March 29, 2012 26:lb741

Rickards CA, **Moralez G**, Romero SA, Cooke WH, Ryan KL, Hinojosa-Laborde C, Lurie KG, and Convertino VA. The influence of oscillations in arterial pressure and cerebral blood velocity on tolerance to hypovolaemia - a review. Presented at Cerebral haemodynamics: Measurement and Management Conference, Imperial College, London England. July, 2011.

Cooke WH, **Moralez G**, and Cox P. Thermographic imaging during simulated uncontrtolled hemorrhage in humans. *Medicine and Science in Sports and Exercise.*

43(5) S448, 2011.

Romero SA, Jacuqez JD, **Moralez G**, Fogt DL, and Cooke WH. Arterial pulse wave velocities are unchanged following 12 weeks of circuit weight training. *Medicine and Science in Sports and Exercise*. 43(5) S445, 2011.

Lara J, **Moralez G**, Barrera C, Martinez A, and Cooke WH. No influence of ovarian hormones on cerebrovascular responses to the Valsalva maneuver. *International Journal of Exercise Science*. 2, Article 28, 2010.

Martinez A, **Moralez G**, Barrera C, Lara J, and Cooke WH. Thermographic imaging to detect reductions of central volume induced by simulated hemorrhage. *International Journal of Exercise Science*. 2, Article 28, 2010.

Barrera C, **Moralez G**, Lara J, Martinez A, and Cooke WH. Non-invasive techniques to track stroke volume during simulated uncontrolled hemorrhage. *International Journal of Exercise Science*. 2, Article 28, 2010.

Sanborn ER, Romero SA, , **Moralez G**, Jacquez JD, , Fogt DL and Cooke WH. Ovarian hormones and cerebral hemodynamics during upright tilt. *International Journal of Exercise Science*. 2, Article 20, 2009.

Jacquez JD, Romero SA, Sanborn ER, **Moralez G**, Liu DD, Fogt DL, and Cooke WH. Arterial pulse wave velocities are unchanged following 12 weeks of circuit weight training. *International Journal of Exercise Science*. 2, Article 12, 2009.

Romero SA, Sanborn ER, **Moralez G**, and Cooke WH. Effects of hypovolemia on cerebral blood velocity and autoregulation during uprignt tilt: Implications for post-spaceflight orthostasis. *International Journal of Exercise Science*. 2, Article 8, 2009.

Ryan KL, Rickards CA, Muniz GW, **Moralez G**, and Convertino VA. Interindividual variability in heart rate variability (HRV) and complexity (HRC) measurements *FASEB J*. 22: 1229.3 2008.

Rickards CA, Ryan KL, Muniz GW, **Moralez G**, and Convertino VA. How many heart beats are enough? A systematic assessment of data length reduction on measures of heart rate variability and complexity *FASEB J*. 22: 1229.1 2008.

Oral Presentations

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Awards

2016 University of North Texas Health Science Center, Dean's Award for Scholarly Excellence in Research Nominee, Selected by the Committee for Graduate studies.

2016 Young Investigator Award from Society for Experimental Biology and Medicine

2016 The American Physiological Society (APS), Caroline tum Suden/Francis A. Hellebrandt Professional Opportunity Award

2016 University of North Texas Health Science Center, Graduate School of Biomedical Sciences, Graduate Student Travel Award

2016 University of North Texas Health Science Center, Graduate Student Association Travel Scholarship

2015 Texas Chapter of American College of Sports Medicine (TACSM) Student Research Development Grant (SRDA- Doctoral) *"The role of central and systemic reactive oxygen species production in the exercise induced resetting of the atrial baroreflex"*

2014 The American Physiological Society (APS) Minority Travel Fellowship Award to attend Experimental Biology 2014 *"Effects of antioxidants on cerebrovascular hemodynamics during moderate and high intensity exercise"*

2013 The American Physiological Society (APS) Minority Travel Fellowship Award to attend Experimental Biology 2013*"Effects of inspiratory resistance on cerebral blood velocity during orthostasis with dehydration"*

2010 Texas Chapter of American College of Sports Medicine (TACSM) Student Manuscript Award Winner. *"Effects of dehydration on cerebrovascular control during standing after heavy resistance exercise"*

2009 Texas Chapter of American College of Sports Medicine (TACSM) Student Research Development Grant (SRDA- Masters) *"Effects of Dehydration on Cerebral Blood Velocity and Autoregulation during Standing after Heavy Resistance Exercise"*

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LIST OF ABBREVIATIONS

Ang II; Angiotensin II

AOX; antioxidant cocktail

ABR; arterial baroreflex

ABP; arterial blood pressure

BL; baseline

BBB; blood brain barrier

BF; burst frequency

BI; burst incidence

CAP; Captopril

Q; cardiac output

CBR; carotid baroreflex

CBs; carotid baroreceptors

SAT; carotid sinus saturation pressure

THR; carotid sinus threshold pressure

CC; central command

DF; deferoxamine methanesulfonate salt

DAP; diastolic arterial pressure

DETC; diethyldithiocarbamic acid sodium

ESR; electron spin resonance

ECSP; estimated carotid sinus pressure

EPR; exercise pressor reflex

FR; free radical

GXT; graded exercise test

HR; heart rate

MSNA; muscle sympathetic nerve activity

NP; neck pressure

NS; neck suction

NO•; nitric oxide

NTS; Nucleus Tractus Solitarius

OP; operating point

PSNA; parasympathetic nerve activity

PER; Perindopril

PL; placebo

RVLM; rostral ventral lateral medulla

SV; stroke volume

SNA; sympathetic nerve activity

SAP; systolic arterial pressure

TVC; total vascular conductance

CHAPTER I:

INTRODUCTION

The arterial baroreflex plays a key role in short-term adjustments in blood pressure in response to abrupt changes in blood volume and cardiac output. Mechanoreceptors located in the bifurcation of the carotid sinus and in the aortic arch sense pressure changes by responding to changes in the tension of the arterial wall. Afferent and efferent neural networks involved in the physiological regulation of sympathetic nerve activity and, in turn, vascular resistances converge centrally at the nucleus tractus solitarius (NTS) within the vasomotor center of the brain within the brainstem. At rest when arterial blood pressure (ABP) is elevated, arterial baroreceptors sense the increase in ABP and increase afferent neuronal information transmitted to the NTS, resulting in a reflex mediated increase in parasympathetic nerve activity and decrease in sympathetic nerve activity to elicit a marked bradycardia and a reduction in ABP. In contrast, during dynamic exercise, an elevated arterial pressure coexists with tachycardia which is essential to maintain increased cardiac output in order to provide appropriate delivery of oxygen to working muscles (Mitchell, 1990; Rowell, 1993).

Neural Cardiovascular Adjustments to Exercise

During progressive increases in exercise intensity the autonomic nervous system exerts cardiovascular and hemodynamic adjustments to modulate perfusion pressure in an exercise intensity related manner (Rowell & O'Leary, 1990). In 1981, Melcher and Donald developed a surgically isolated carotid baroreceptor model in exercising canines and established that the arterial baroreflex (ABR) stimulus-response function curves were reset rightward and upward

during treadmill running exercise without any change in arterial baroreflex sensitivity (Melcher & Donald, 1981). Later, Rowell and O'Leary (1990), proposed a hypothetical model of ABR "resetting" during exercise. Their model proposed that at exercise onset and with subsequent increases in exercise intensity the feed forward mechanism (input from the higher motor centers) known as central command (CC) resets the Operating Point (OP) pressure of the arterial baroreflex stimulus-response curve relationship laterally to higher OP pressures by acting centrally on the neuron pool receiving the baroreceptor afferents. At the same time, a vertical shift in the baroreflex function curves was affected by the group III & IV muscle afferents, or the exercise pressor reflex (EPR), which increased sympathetic nerve activity without changing the OP pressure because the stimulus acts only on the efferent arm of the reflex and not on the central neurons controlling the reflex. The resulting vector of the horizontal and vertical shifts predicted by the modeled increases in sympathetic activity was proposed to result in an upward and rightward "resetting" of the OP pressure around which the ABR operated (figure 1). Since Rowell and O'Leary (1990), proposed this hypothetical model of ABR "resetting" during exercise, numerous investigations in human subjects have confirmed that, indeed, activation of CC and the EPR are essential for the progressive physiological resetting of the reference "OP pressures" of the ABR in direct relation to the progressive increase in exercise intensity (Potts *et al.*, 1993; Bishop, 1994; Norton *et al.*, 1999b; McIlveen *et al.*, 2001; Fadel & Raven, 2012) confirming Melcher & Donald's findings from (1981).

Assessment of Baroreflex-Mediated Vascular Responses in Humans

The assessment of baroreflex-mediated cardiac and vascular responses in humans utilizing a neck pressure (NP)-neck suction (NS) technique in which the open-loop stimulus-response relationship of carotid-cardiac and carotid-vasomotor baroreflex control has been demonstrated

to shift rightward and upward in direct relation to dynamic exercise intensity (Potts *et al.*, 1993; Norton *et al.*, 1999a; Fadel *et al.*, 2001). This technique is performed by use of a customized computer controlled variable pressure neck collar fitted onto the anterior 2/3 of the subject's neck (Pawelczyk & Raven, 1989; Potts *et al.*, 1993). The collar provides an airtight chamber which encompasses the anatomical location of the carotid sinus area that is verified by ultrasound imaging. When prompted, the computer software initiates a positive (neck pressure, NP) or negative (neck suction, NS) pressure pulse for 5 seconds. The positive or negative chamber pressure within the collar transmits 89% of the NP and 83% of the NS, respectively, to the extramural carotid tissues (Querry *et al.*, 2001b), which is sensed by the carotid baroreceptors (CBs) as changes in carotid artery transmural pressure (Potts *et al.*, 1993). The NP simulates hypotension and the NS simulates hypertension and both simulations are sensed by the carotid baroreceptors (CBs).The CBs can respond to alterations in mean arterial pressure (MAP) by affecting three primary physiological variables HR, stroke volume (SV), and total vascular conductance (TVC) in accordance with the following equation (Ogoh *et al.*, 2003):

$$MAP = (HR \times SV) / TVC$$

Ogoh *et al.* (2003) identified that alterations in vasomotion is the primary means by which the CBR regulates blood pressure during mild to heavy exercise. Resetting of the ABR enables the observed parallel increases in HR and ABP with progressive increases in exercise intensity. The functional resetting of ABR control of heart rate (HR), MSNA, and MAP are, however, not accompanied by changes in ABR maximal gain or baroreflex sensitivity (BRS) (Potts *et al.*, 1993; Norton *et al.*, 1999a; Fadel *et al.*, 2001). Additionally, a number of studies, which have examined the HR reflex resetting during progressive increases in exercise intensity, indicated that HR reflex sensitivity was diminished with increases in exercise intensity (Ogoh *et al.*, 2003;

Ogoh *et al.*, 2005). That said, since Ogoh *et al.* (2003) established that alterations in vasomotor control of blood vessels are the primary means by which the CBR regulates blood pressure during mild to heavy exercise, ***the current investigation only focused on*** the exercise related rightward and upward resetting of the ABR-OP pressure and the reflex control of central sympathetic outflow and not on the functional resetting of ABR control of HR.

Assessment of baroreflex-mediated vascular responses in humans utilizing the NP/NS technique over the past 27 years has established that in humans, CC and/or the EPR are required for physiologic resetting of the arterial baroreflex (ABR) during exercise (Pawelczyk & Raven, 1989; Potts *et al.*, 1993; Norton *et al.*, 1999a; Gallagher *et al.*, 2001; Querry *et al.*, 2001a; Fadel & Raven, 2012; Fisher *et al.*, 2015). In addition, these studies established that cardiopulmonary baroreceptors (mechanoreceptors within the cardiac walls (Paintal, 1973)) sense changes in central blood volume at rest and during exercise and reflexly modulate central SNA outflow and the OP pressure of the ABR (Volianitis *et al.*, 2004; Ogoh *et al.*, 2006; Ogoh *et al.*, 2007)

While the neural mechanisms involved in exercise induced ABR resetting have been established (Fadel, 2008; Raven, 2012; Michelini *et al.*, 2015), the signaling mediators of this well-established phenomenon remain unknown. Animal data has identified that nitric oxide (NO•) in the CNS acts as a sympatho-inhibitory molecule (Patel *et al.*, 2001; Schultz, 2009; Gao *et al.*, 2011) and there is a growing body of evidence that indicates that central NO• is scavenged by centrally generated free radicals (FR) (Paton & Waki, 2009; Waki *et al.*, 2011; Leal *et al.*, 2012; Leal *et al.*, 2013). Furthermore, during dynamic exercise increases in centrally generated FRs are formed during exercise intensity-related oxidative metabolism and central Ang II linked FR production (Fallo, 1993; Bailey *et al.*, 2004; Shim *et al.*, 2008; Powers *et al.*, 2010; Bailey *et al.*, 2011; Powers *et al.*, 2016). Therefore, a logical molecular signaling candidate involved in the

physiological mechanisms of exercise-induced ABR resetting is the increased production of FRs that occurs during dynamic exercise.

Nitric Oxide and Free Radicals as modulators of central sympathetic outflow

NO• is synthesized in several types of cells by a trio of isoenzymes termed nitric oxide synthases (NOS) (Prast & Philippu, 2001). Cells such as endothelial cells (eNOS), macrophages/inducible (iNOS) and neurons (nNOS) produce NO by oxidizing a guanidino nitrogen of L-arginine, utilizing molecular oxygen and NADPH as co-substrates (Mayer *et al.*, 1991; Lohse *et al.*, 1998). Within the CNS, Garthwaite *et al.* (1988) was the first to suggest that NO (known as endothelium derived relaxing factor (EDRF) at the time) played a role as a messenger molecule. Since then, a number of investigations have identified that indeed NO is a modulator of neuronal function (Hanbauer *et al.*, 1992; Lonart *et al.*, 1992; Prast *et al.*, 1992; Prast & Philippu, 1992b, a). In the brainstem, nNOS-containing neurons are found in the periaqueductal gray, parabrachial nucleus, raphe nuclei, NTS, the dorsal motor nucleus, the nucleus ambiguous (NA), and the caudal and rostral regions of the ventral lateralmedulla (VLM) (Krukoff, 1999; Yang *et al.*, 1999; Schultz, 2009). Thus, nNOS is ideally positioned to serve as a neuromodulator evoking or enhancing both excitatory and inhibitory neuronal signaling within the brainstem (Wang *et al.*, 2007). However, studies have identified that NO• acts as a sympatho-inhibitory molecule in the CNS (Aslan *et al.*, 2001; Patel *et al.*, 2001; Schultz, 2009; Gao *et al.*, 2011). Specifically, central sympathetic outflow is inhibited by NO within the NTS and RVLM (Kishi *et al.*, 2001; Zucker *et al.*, 2001; Chan *et al.*, 2003). Furthermore, studies performed in conscious animals support a general consensus that central NO restrains sympathetic outflow and facilitates parasympathetic output, particularly when feedback from the baroreceptor reflex is controlled (Patel *et al.*, 2001; Zucker *et al.*, 2001). In addition to the animal investigations, NOS inhibition in humans increases

muscle sympathetic nerve activity when changes in arterial pressure are buffered (Sartori *et al.*, 2005). During exercise, contracting muscles produce NO peripherally (Balon & Nadler, 1994; Radak *et al.*, 1999) and a number of studies have identified that centrally produced NO buffers the pressor response to activation of the EPR (Li & Potts, 2001; Smith *et al.*, 2005; Leal *et al.*, 2012; Murphy *et al.*, 2013).

Free Radicals

Free radicals (FR) and other reactive oxygen species (ROS) are generated either from normal essential metabolic or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial sources (Lobo *et al.*, 2010). For the purpose of this investigation we will focus on FRs, particularly superoxide (O_2•-), which is produced during metabolic processes such as those that occur during exercise (Powers *et al.*, 2010; Bailey *et al.*, 2011; Powers *et al.*, 2016) and activation of the Renin-Angiotensin system (RAS) (Griendling *et al.*, 1994; Zimmerman *et al.*, 2002; Zimmerman *et al.*, 2004; Chan *et al.*, 2005).

Increased O_2•- formation, results from increased electron transport chain flux associated with increased metabolism in contracting skeletal muscles (Powers *et al.*,2010; Bailey *et al.*,2011). Reid *et al.* (1992a) were the first to report that contracting skeletal muscle fibers release O_2•- intracellularly (Reid *et al.*, 1992a) and extracellularly (Reid *et al.*, 1992b) in rodents. O'Neill *et al.* (1996) later confirmed Reid *et al.*'s1992 findings in the contracting skeletal muscle of cats and established that the rate of radical production increases in proportion to the percentage of maximal force. Utilizing electron paramagnetic resonance (EPmR) Bailey and colleagues were the first investigators to provide direct evidence for intramuscular free radical accumulation

following exercise in humans (Bailey *et al.*, 2007) confirming that moderate to severe exercise is an established method of inducing an oxidative stress.

In addition to the metabolic production of FRs during skeletal muscles contractions FRs can also be produced by activation of the RAS which is active during exercise (Galbo, 1986; Fallo, 1993). Specifically, Angiotensin II (Ang II) induces O_2•- formation by activating NAD(P)H oxidase peripherally (Griendling *et al.*, 1994) and centrally (Zimmerman *et al.*, 2002; Chan *et al.*, 2005; Zucker, 2006). An exaggerated blood pressure response to exercise has been associated with an augmented rise of Ang II during exercise in human subjects (Shim *et al.*, 2008).

All of these processes resulting in FR production modify central sympathetic neural outflow (Zimmerman *et al.*, 2004; Campese *et al.*, 2005; Han *et al.*, 2005). For example, NO can be depleted by its reaction with the O_2•- to generate peroxynitrite resulting in disinhibition within the NTS and RVLM and, subsequently, increased central sympathetic outflow. Indeed animal experiments have demonstrated that FR production scavenges NO within the NTS and RVLM enabling an increased central sympathetic neural outflow (Aslan *et al.*, 2001; Waki *et al.*, 2008; Paton & Waki, 2009; Schultz, 2009; Fisher & Fadel, 2010) . Furthermore, it is well established that central and peripheral produced FRs are involved in blood pressure regulation and that FRs are linked to Ang II dependent hypertension (Grobe *et al.*, 2008; Datla & Griendling, 2010). When the Ang II linked FR production becomes magnified at rest, neurogenic hypertension ensues (Waki *et al.*, 2008; Paton & Waki, 2009; Waki *et al.*, 2011). Identifying the physiological mechanisms for exercise-induced ABR resetting in healthy individuals and the impact of increasing dynamic exercise intensities on these mechanisms will have major clinical significance. For example, clarifying the central and peripheral contributions of Ang II and FR production and their involvement in arterial baroreflex resetting will not only advance our

understanding regarding the physiological mechanisms governing reflex cardiovascular control during exercise, but also provide important insight into the patho-physiological mechanisms of impaired autonomic regulation of exercise induced hypertension (EIHt).

In this investigation FR production will be suppressed by using antioxidants and angiotensin converting enzyme inhibitors (ACEi). A unique feature of this project is the repeated measurements of postganglionic muscle sympathetic nerve activity in the radial nerve in humans during repeated bouts of dynamic leg exercise. Another innovation in the proposed research is the comparison of substances which act globally (both centrally and peripherally) vs. those which only act peripherally.

HYPOTHESIS:

We hypothesize that free radicals are the signaling molecules that enable arterial baroreflex resetting during exercise in healthy volunteers. We expect that due to the permeability kinetics of the globally acting pharmacological agents they will produce a greater attenuation of the exercise-induced resetting of ABR control of MAP and MSNA than observed with the agents acting only peripherally.

The proposed investigation will address the global hypothesis that: Central production of angiotensin II and its concomitant FR production and neural-metabolic production of FR drives the intensity related arterial baroreflex resetting during exercise in healthy volunteers. Blockade of FR production will be accomplished by using antioxidants and ACEi. The outcome of this project will identify the physiological mechanism of arterial baroreflex resetting during exercise and as a consequence will provide insight into the patho-physiological mechanisms of impaired autonomic regulation of cardiovascular responses such as EIHt. It may also pave the way to

develop treatments for patients with exercise-induced hypertension who wish to obtain the benefits of exercise. To address this global hypothesis, the following specific aims are focused on separating the effect of central and peripheral FR accumulation on ABR resetting that will be tested in young, normotensive human subjects:

Specific Aim 1: To test the hypothesis that the combined central and peripheral exercise intensity related production of FRs plays a pivotal role in the exercise related resetting of the arterial baroreflex. This aim will be tested by pharmacological inhibition of ROS production using a combined central and peripheral acting antioxidant cocktail of Co-enzyme Q, Vitamin E and Vitamin C compared to a placebo.

Specific Aim 2: To test the hypothesis that acute blockade of the central and peripheral production of Ang II using effective equivalent doses of Perindopril compared to a selective peripheral blockade of Ang II production using Captopril will reduce sympathetic nerve activity and arterial baroreflex resetting more during increases in exercise intensity than during placebo conditions.

A unique feature of this project is the implementation of radial nerve microneurography during repeated bouts of dynamic leg exercise. In order to address exercise-induced physiological blood volume shifts, the exercise performed must be dynamic and upright. Recording sympathetic nerve traffic in the radial nerve of the upper arm allows both legs to be used for exercise and thereby, more closely mimic exercise intensity induced changes in central blood volume (Ogoh *et al.*, 2007) that occur during human activities of daily living. Another innovation in the proposed research will be the comparison of ACEi which act globally (both central and peripheral) against those which only act peripherally (Zimmerman *et al.*, 2002;

Campese *et al.*, 2004; Lee *et al.*, 2009; Villapol & Saavedra, 2015). We anticipate that the findings of these investigations will delineate the physiologic mechanism involved in baroreflex resetting during exercise and provide evidence for investigating the pathophysiologic mechanisms of impaired autonomic regulation of cardiovascular responses to exercise.

Experimental design

Recruitment of Healthy Subjects (for Specific Aims 1 & 2) *Subject Criterion*: Healthy volunteers aged 18-35 were recruited to participate in these investigations. All subjects were free from known cerebrovascular/cardiovascular diseases, under no current medication and were not using any tobacco products (including chewing tobacco). Written informed consent was obtained from all subjects. Subjects were asked to abstain from caffeine, exercise, alcohol, and foods high in antioxidants (e.g. berries, beans, orange juice) 24 hours prior to all visits to the laboratory. Subjects taking multivitamins regularly were instructed to refrain from its use for at least 15 days before they were scheduled for experimentation. Only women with a documented negative pregnancy test within 24 hours prior to each study period were included. Due to the potential effect of estrogen and progesterone on autonomic control, all female subjects not on hormonal contraception were tested in the early follicular (low hormone) phase of their menstrual cycle; female subjects on hormonal contraception were tested during the low hormone or placebo phase of their contraception cycle (i.e., days 1-4).

Orientation Day (for Specific Aims 1 & 2): Each subject was invited to the laboratory in order to complete the consent process, including an explanation of the experimental techniques to be used and risks involved. Subsequently, the participants provided their written consent on the Institutional Review Board (IRB) approved informed consent paperwork (IRB# 2014-062 &

#2014-089). Following completion of the consent process, the subjects were asked to complete a General Health History Questionnaire to provide us with preliminary information pertaining to their health status. Each subject performed an orientation protocol to familiarize them with the laboratory, personnel and equipment to be used during the experimental protocol in order to reduce any potential 'white-coat' effects on measured variables during the experimental protocol. Following completion of the orientation each subject performed a graded exercise test (GXT) on a 70° back supported semi-recumbent cycle ergometer to determine their exercise tolerance and workload for steady state exercise at HRs of 120 beats/min (EX120) and 150 beats/min (EX150). In addition, each subject practiced wearing the neck collar for assessment of baroreflex sensitivity, and performed 2-3 trials of NP/NS at rest and during mild exercise. After the GXT protocol, subjects were then assigned in a randomized cross-over design (known only to the PI) to the Experimental Treatments:

Specific Aim 1: i) Placebo (PL); or ii) Anti-oxidant Cocktail (AOX)

Specific Aim 2: i) Placebo (PL); ii) Captopril (CAP); iii) Perindopril (PER).

Experimental Protocol for Specific Aim 1: The experiment was comprised of three visits to the laboratory. The first visit was the Orientation Day (approx.1 hour) and the second and third visits were Experimental Days (a maximum of 4 hours each). Experimental protocols were separated by at least one week during which the subjects were instructed to maintain their normal exercise and diet regimen. Once subjects successfully completed the orientation day they were invited to participate in the experimental protocol performed at the same time of day for each subject. After ingestion of either the PL or AOX capsules each subject was instrumented for the measurement

of HR, ABP, MSNA and assessment of baroreflex function curves using our customized NP/NS protocol; a venous (ante-cubital vein) catheter was also placed for blood sampling, (details to follow).

Absorption Period for Specific Aim 1: The subject ingested the antioxidant cocktail (AOX) or placebo (PL), according to their experimental treatment assignment. Treatment assignment will be coded and blinded to the subject. The time between oral ingestion of PL or AOX and exercise initiation was ~60min, as this is the time needed for the peak plasma activity, as determined by previous studies (Chang *et al.*, 1996; Levine *et al.*, 1996; Lee *et al.*, 2009). ***The AOX Cocktail*** was based on the work of (Lee *et al.*, 2009) who observed that their antioxidant formulation mitigated intermittent hypoxia induced ventilation adaptations in humans, which in animal models is demonstrated to be associated with oxidative stress (Prabhakar *et al.*, 2007). In this set of experiments, we compared the efficacy of this cocktail preparation in reducing exercise induced oxidative stress. Similar to the Lee *et al.* (2009) protocols, we prepared an antioxidant cocktail of 150 IU Vitamin E (α- tocopherol), 100 mg Co-enzyme Q10 (Ubiquinone USP Grade, Qunol™, Ultra CoQ10, Fairfield, NJ 07004), and 2 g Vitamin C (Ascorbic Acid, REXALL Sundown, INC. Boca Raton, FL 33487). The hydrophilic AA scavenges FRs in the plasma and cytosol of the peripheral (i.e. not central nervous system) compartment; CoQ10 and Vitamin E are lipophilic antioxidants that reduce oxidative stress both peripherally and centrally (i.e. by crossing the BBB).

Exercise protocol (for Specific Aims 1 & 2): The subjects were positioned within a customized box enclosing a stationary cycle ergometer in a 70^0 back supported semi-recumbent position with the legs extended parallel with the floor and feet resting on the pedals. The subjects rested in the semi-recumbent position during instrumentation for a minimum 10-min or more without

disturbance to establish pre-exercise baseline (BL) values and a blood sample was obtained. The exercise protocol consisted of two stages: 1) **Stage EX120** (exercise at HR of 120 beats/min heavy- intensity), 2) **Stage EX150** (exercise at HR of 150 beats/min very heavy intensity) (Åstrand & Rodahl, 1977, 1986). At the start of EX120 the pedal frequency and the intensity of exercise were progressively increased to a maximum of 60 rpm over the next 5 min to achieve a constant workload at a HR of 120 beats/min. After attaining steady-state circulatory responses (after ~ 5 min at each workload), exercise data were collected for 5 min. Upon completion of the subject's exercising steady-state baseline recording a blood sample was obtained and was followed by the carotid baroreflex's control of mean arterial pressure (MAP) was assessed (Potts *et al.,* 1993; Norton *et al.,* 1999b; Ogoh *et al.,* 2003). The two exercise workloads were performed continuously for approximately 20 min each.

Protocol for Specific Aims 2: This experiment required subjects to visit our laboratory on four different occasions. The first visit was the **Orientation Day** and the three subsequent visits were **Experimental Days**. Experimental protocols were separated by at least one week during which the subjects were instructed to maintain their habitual exercise and diet regimen. Once subjects successfully completed the orientation day (previously described) they were invited to participate in the experimental protocol performed at the same time of day for each subject. After ingestion of either the PL, CAP, or PER capsules, the subject was instrumented for the data collection of HR, ABP, MSNA and measurement of carotid baroreflex function curves using our NP/NS protocol, and a venous (ante-cubital vein) catheter was placed for blood sampling.

Dosages and Absorption Period for Specific Aim 2:

Comparison doses for the ACE inhibitors were determined by our board certified cardiologist consultant and were set as the acute dose required for approximately equal changes in blood pressure in clinical applications based on previous studies (Lees & Reid, 1987; Agabiti-Rosei *et al.*, 1992; Jankowski *et al.*, 1995; Chik *et al.*, 2010). Furthermore, single dose studies in healthy human subjects have identified that plasma angiotensin II concentration are reduced by 31% with both 25mg CAP measured at 1 hour post-ingestion (Hollenberg *et al.*, 1981) and 4mg of PER measured at hour 4 post-ingestion (Bussien *et al.*, 1986). In rare instances anaphylaxis has been observed following ACEi, hence, our counterbalanced order always presented Captopril ahead of Perindopril because the time for Captopril to achieve peak blood concentrations occurs in the 1hr the subject is being instrumented in the laboratory.

Table 1. Angiotensin converting enzyme inhibitors

Experimental Treatment	**ACE inhibitors**	**Time required for peak Blood Concentration (Single Dose)**	**Dose**
PL	Placebo		Sugar pill
CAP	Captopril	1 Hour	25 mg
PER	Perindopril	4-5 Hours	4 mg

Similar reductions in peripheral plasma angiotensin II concentration helped us identify the main site of action of Ang II. Peripherally circulating Ang II inhibits baroreflex activity through interactions with AT_1 receptors in the circumventricular organs (CVOs) (Sanderford & Bishop, 2002; Tan *et al.*, 2007).The subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) are CVOs which lack a functional BBB and are sensitive to circulating Ang II (Osborn *et al.*, 2007). The SFO and OVLT innervate the median preoptic nucleus (MnPO)

(Johnson & Gross, 1993) which projects to the PVN (Stocker & Toney, 2005) and modulate sympathetic outflow through the NTS and RVLM. Therefore, by administering ACEi doses that attenuated the rise in circulating Ang II to the same extent during exercise any changes in sympathetic outflow could be contributed to the lipid soluble agent acting centrally on endogenous Ang II (Figure 2).

Experimental Measurements:

Non-invasive arterial blood pressure: a photoplethysmography blood pressure monitor was used to record the arterial pressure waveform continuously (Finometer™, Finapres Medical Systems, Amsterdam, The Netherlands.

Neck pressure/neck suction: Manipulation of the arterial carotid-MAP baroreflex function curves were obtained by using a customized computer controlled variable pressure neck collar fitted onto the anterior 2/3 of the subject's neck (Pawelczyk & Raven, 1989; Potts *et al.*, 1993). The collar provides an airtight chamber which encompasses the location of the carotid sinus area, which is verified by ultrasound imaging. The computer software initiates a positive (neck pressure, NP) or negative (neck suction, NS) pressure pulse for 5 sec. Pressures were randomly applied between +40 and -60 Torr in 20 Torr increments. Within the collar 89% of the NP and 83% of the NS were transmitted to the tissue surrounding the carotid baroreceptors, respectively. The carotid baroreceptors sensed the NP/NS induced changes in tissue pressure as changes in transmural pressure across the carotid sinus (Potts *et al.*, 1993). Physiologically NP simulates hypotension and NS simulates hypertension that is sensed by the carotid baroreceptors. Beat-to-beat measurements MAP were analyzed to determine the responses to NP/NS stimulation. Carotid baroreflex (CBR) function curves were constructed using the inverse logistic function

(Kent *et al.*, 1972) without correcting for degree of transmission. Modeling of peak ABP response to individual NPs and NSs will be performed to determine carotid-–vasomotor baroreflex function curves (Potts *et al.*, 1993; Ogoh *et al.*, 2003). Threshold and saturation calculations were determined by applying a correction of the exponential developed by McDowall & Dampney (McDowall & Dampney, 2006) to the Kent logistic function algorithm.

Venous catheter placement and blood sampling: A catheter was placed into an antecubital vein to obtain blood samples. The blood samples were used to measure FR using superoxide-dependent electron spin resonance (ESR) spectroscopy under the direction of our collaborator Matthew Zimmerman Ph.D. at the University of Nebraska Medical Center.

Muscle Sympathetic Nerve Activity (MSNA): Postganglionic multiunit muscle sympathetic nerve activity (MSNA) was recorded from the radial nerve at the spiral groove using ultrasound-guided (Curry & Charkoudian, 2011) standardized microneurography techniques (Hagbarth & Eklund, 1968; Vallbo & Hagbarth, 1968; Wallin *et al.*, 1994). The neural activity was band-pass filtered (100–2000 Hz), and integrated (time constant, 0.1 s) to obtain mean voltage neurograms (Iowa Biosystems, Iowa, USA). Neural recordings were accepted as muscle SNA when spontaneous burst discharges were synchronized with heart beat and enhanced by apnea, but were unaffected by cutaneous touch or arousal stimuli (Vallbo *et al.*, 1979). Neurograms were subsequently imported into an analysis program (WinCPRS, Absolute Aliens, Turku, Finland) that has the capability of detecting bursts of MSNA. The software detects a burst of MSNA based on two primary criteria: i) pulse synchronous spontaneous bursts with signal-to-noise ratios of approximately 3 :1; and ii) reflex latencies from preceding R-waves of approximately 0.9 s (Wallin et al., 1994; Cooke et al., 2009). Subsequently, each was file was manually checked

for the computerized burst detection results. MSNA was expressed as bursts per minute, i.e. burst frequency (BF) and as bursts per 100 heart beats, i.e. burst incidence (BI).

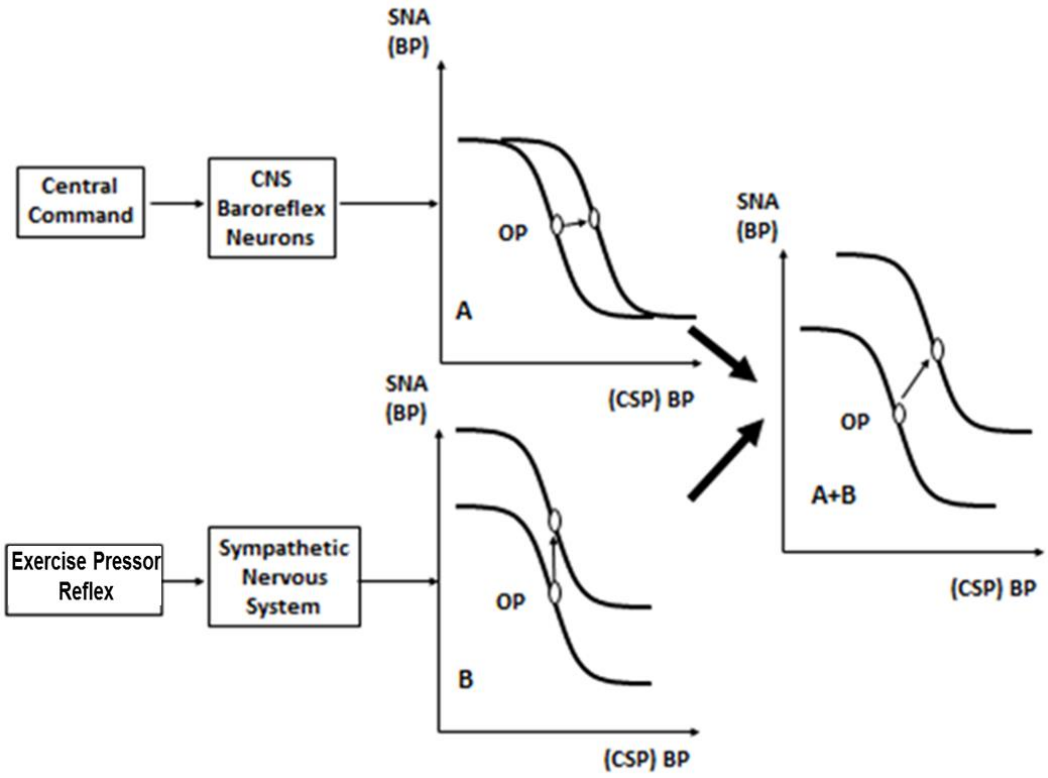

Figure 1. Modified Schematic of Rowell and O'Leary's (1990), hypothetical model of ABR "resetting" during exercise. Central Command resets the operating point (OP) of the arterial baroreflex stimulus-response curve relationship laterally (A) while at the same time the Exercise Pressor Reflex causes vertical shift in the OP (B). The resulting vector is a horizontal and vertical shift of the arterial baroreflex stimulus-response curve (A+B).

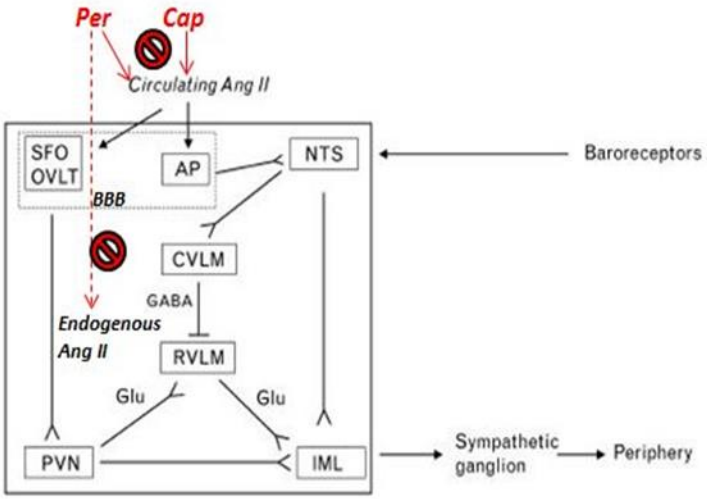

Figure 2. Proposed schematic of the use of two different lipid soluble pharmacological agents that differentially block circulating and endogenous (central) angiotensin II. Modified from Dupont and Brouwers (Dupont & Brouwers, 2010). Captopril (CAP), Perindopril (PER), Subfornical Organ (SFO), Organum Vasculosum of the Lamina Terminalis (OVLT), Area Postrema (AP), paraventricular nucleus (PVN), Caudal Ventrolateral Medulla (CVLM), Rostral ventrolateral medulla (RVLM), Nucleus Tractus Solitarius (NTS), Intermediolateral Cell Column (IML), gamma-aminobutyric acid (GABA), and Glutamic acid (Glu).

REFERENCES

Agabiti-Rosei E, Ambrosioni E, Finardi G, Folino P, Gambassi G, Malini P, Marchesi E, Muiesan ML, Semplicini A & Pessina AC. (1992). Perindopril versus captopril: efficacy and acceptability in an Italian multicenter trial. *Am J Med* **92,** 79S-83S.

Aslan M, Ryan TM, Adler B, Townes TM, Parks DA, Thompson JA, Tousson A, Gladwin MT, Patel RP, Tarpey MM, Batinic-Haberle I, White CR & Freeman BA. (2001). Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Natl Acad Sci U S A* **98,** 15215-15220.

Åstrand P-O & Rodahl K. (1977). *Textbook of work physiology : physiological bases of exercise*. McGraw-Hill, New York.

Åstrand P-O & Rodahl K. (1986). *Textbook of work physiology : physiological bases of exercise*. McGraw-Hill, New York ; London.

Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, Ogoh S, Ainslie PN, Lucchesi C, Rockenbauer A, Culcasi M & Pietri S. (2011). Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. *Experimental physiology* **96,** 1196-1207.

Bailey DM, Lawrenson L, McEneny J, Young IS, James PE, Jackson SK, Henry RR, Mathieu-Costello O, McCord JM & Richardson RS. (2007). Electron paramagnetic spectroscopic evidence of exercise-induced free radical accumulation in human skeletal muscle. *Free Radic Res* **41,** 182-190.

Bailey DM, Young IS, McEneny J, Lawrenson L, Kim J, Barden J & Richardson RS. (2004). Regulation of free radical outflow from an isolated muscle bed in exercising humans. *American journal of physiology Heart and circulatory physiology* **287,** H1689-1699.

Balon TW & Nadler JL. (1994). Nitric oxide release is present from incubated skeletal muscle preparations. *J Appl Physiol (1985)* **77,** 2519-2521.

Bishop VS. (1994). "Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise". *J Appl Physiol (1985)* **77,** 491-492.

Bussien JP, d'Amore TF, Perret L, Porchet M, Nussberger J, Waeber B & Brunner HR. (1986). Single and repeated dosing of the converting enzyme inhibitor perindopril to normal subjects. *Clin Pharmacol Ther* **39,** 554-558.

Campese VM, Shaohua Y & Huiquin Z. (2005). Oxidative stress mediates angiotensin II-dependent stimulation of sympathetic nerve activity. *Hypertension* **46,** 533-539.

Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z & Chiu J. (2004). Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* **287,** H695-703.

Chan SH, Hsu KS, Huang CC, Wang LL, Ou CC & Chan JY. (2005). NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38 mitogen-activated protein kinase in the rostral ventrolateral medulla. *Circ Res* **97,** 772-780.

Chan SH, Wang LL & Chan JY. (2003). Differential engagements of glutamate and GABA receptors in cardiovascular actions of endogenous nNOS or iNOS at rostral ventrolateral medulla of rats. *Br J Pharmacol* **138,** 584-593.

Chang T, Benet LZ & Hebert MF. (1996). The effect of water-soluble vitamin E on cyclosporine pharmacokinetics in healthy volunteers. *Clin Pharmacol Ther* **59,** 297-303.

Chik Z, Basu RC, Pendek R, Lee TC & Mohamed Z. (2010). A bioequivalence comparison of two formulations of rifampicin (300- vs 150-mg capsules): An open-label, randomized, two-treatment, two-way crossover study in healthy volunteers. *Clin Ther* **32,** 1822-1831.

Cooke WH, Rickards CA, Ryan KL, Kuusela TA & Convertino VA. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol* **587,** 4987-4999.

Curry TB & Charkoudian N. (2011). The use of real-time ultrasound in microneurography. *Auton Neurosci* **162,** 89-93.

Datla SR & Griendling KK. (2010). Reactive oxygen species, NADPH oxidases, and hypertension. *Hypertension* **56,** 325-330.

Dupont AG & Brouwers S. (2010). Brain angiotensin peptides regulate sympathetic tone and blood pressure. *J Hypertens* **28,** 1599-1610.

Fadel PJ. (2008). Arterial baroreflex control of the peripheral vasculature in humans: rest and exercise. *Med Sci Sports Exerc* **40,** 2055-2062.

Fadel PJ, Ogoh S, Watenpaugh DE, Wasmund W, Olivencia-Yurvati A, Smith ML & Raven PB. (2001). Carotid baroreflex regulation of sympathetic nerve activity during dynamic exercise in humans. *American journal of physiology Heart and circulatory physiology* **280,** H1383-1390.

Fadel PJ & Raven PB. (2012). Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. *Experimental physiology* **97,** 39-50.

Fallo F. (1993). Renin-angiotensin-aldosterone system and physical exercise. *J Sports Med Phys Fitness* **33,** 306-312.

Fisher JP & Fadel PJ. (2010). Therapeutic strategies for targeting excessive central sympathetic activation in human hypertension. *Experimental physiology* **95,** 572-580.

Fisher JP, Young CN & Fadel PJ. (2015). Autonomic adjustments to exercise in humans. *Compr Physiol* **5,** 475-512.

Galbo H. (1986). The hormonal response to exercise. *Diabetes Metab Rev* **1,** 385-408.

Gallagher KM, Fadel PJ, Stromstad M, Ide K, Smith SA, Querry RG, Raven PB & Secher NH. (2001). Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *J Physiol* **533,** 871-880.

Gao J, Zhang H, Le KD, Chao J & Gao L. (2011). Activation of central angiotensin type 2 receptors suppresses norepinephrine excretion and blood pressure in conscious rats. *Am J Hypertens* **24,** 724-730.

Garthwaite J, Charles SL & Chess-Williams R. (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336,** 385-388.

Griendling KK, Minieri CA, Ollerenshaw JD & Alexander RW. (1994). Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* **74,** 1141-1148.

Grobe JL, Xu D & Sigmund CD. (2008). An intracellular renin-angiotensin system in neurons: fact, hypothesis, or fantasy. *Physiology* **23,** 187-193.

Hagbarth KE & Eklund G. (1968). Motor effects of muscle vibration in spasticity, rigidity and cerebellar disorders. *Electroencephalogr Clin Neurophysiol* **25,** 407.

Han Y, Zhang Y, Wang HJ, Gao XY, Wang W & Zhu GQ. (2005). Reactive oxygen species in paraventricular nucleus modulates cardiac sympathetic afferent reflex in rats. *Brain Res* **1058,** 82-90.

Hanbauer I, Wink D, Osawa Y, Edelman GM & Gally JA. (1992). Role of nitric oxide in NMDA-evoked release of [3H]-dopamine from striatal slices. *Neuroreport* **3,** 409-412.

Hollenberg NK, Meggs LG, Williams GH, Katz J, Garnic JD & Harrington DP. (1981). Sodium intake and renal responses to captopril in normal man and in essential hypertension. *Kidney Int* **20,** 240-245.

Jankowski A, Skorek A, Krzysko K, Zarzycki PK, Ochocka RJ & Lamparczyk H. (1995). Captopril: determination in blood and pharmacokinetics after single oral dose. *J Pharm Biomed Anal* **13,** 655-660.

Johnson AK & Gross PM. (1993). Sensory circumventricular organs and brain homeostatic pathways. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **7,** 678-686.

Kent BB, Drane JW, Blumenstein B & Manning JW. (1972). A mathematical model to assess changes in the baroreceptor reflex. *Cardiology* **57,** 295-310.

Kishi T, Hirooka Y, Sakai K, Shigematsu H, Shimokawa H & Takeshita A. (2001). Overexpression of eNOS in the RVLM causes hypotension and bradycardia via GABA release. *Hypertension* **38,** 896-901.

Krukoff TL. (1999). Central actions of nitric oxide in regulation of autonomic functions. *Brain Res Brain Res Rev* **30,** 52-65.

Leal AK, Mitchell JH & Smith SA. (2013). Treatment of muscle mechanoreflex dysfunction in hypertension: effects of L-arginine dialysis in the nucleus tractus solitarii. *Exp Physiol*.

Leal AK, Murphy MN, Iwamoto GA, Mitchell JH & Smith SA. (2012). A role for nitric oxide within the nucleus tractus solitarii in the development of muscle mechanoreflex dysfunction in hypertension. *Experimental physiology* **97,** 1292-1304.

Lee DS, Badr MS & Mateika JH. (2009). Progressive augmentation and ventilatory long-term facilitation are enhanced in sleep apnoea patients and are mitigated by antioxidant administration. *J Physiol* **587,** 5451-5467.

Lees KR & Reid JL. (1987). Haemodynamic and humoral effects of oral perindopril, an angiotensin converting enzyme inhibitor, in man. *Br J Clin Pharmacol* **23,** 159-164.

Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J & Cantilena LR. (1996). Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci U S A* **93,** 3704-3709.

Li J & Potts JT. (2001). NO formation in nucleus tractus solitarii attenuates pressor response evoked by skeletal muscle afferents. *Am J Physiol Heart Circ Physiol* **280,** H2371-2379.

Lobo V, Patil A, Phatak A & Chandra N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* **4,** 118-126.

Lohse MJ, Forstermann U & Schmidt HH. (1998). Pharmacology of NO:cGMP signal transduction. *Naunyn Schmiedebergs Arch Pharmacol* **358,** 111-112.

Lonart G, Wang J & Johnson KM. (1992). Nitric oxide induces neurotransmitter release from hippocampal slices. *Eur J Pharmacol* **220,** 271-272.

Mayer B, John M, Heinzel B, Werner ER, Wachter H, Schultz G & Bohme E. (1991). Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase. *FEBS Lett* **288,** 187-191.

McDowall LM & Dampney RA. (2006). Calculation of threshold and saturation points of sigmoidal baroreflex function curves. *Am J Physiol Heart Circ Physiol* **291,** H2003-2007.

McIlveen SA, Hayes SG & Kaufman MP. (2001). Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol* **280,** H1454-1463.

Melcher A & Donald DE. (1981). Maintained ability of carotid baroreflex to regulate arterial pressure during exercise. *Am J Physiol* **241,** H838-849.

Michelini LC, O'Leary DS, Raven PB & Nobrega AC. (2015). Neural control of circulation and exercise: a translational approach disclosing interactions between central command, arterial baroreflex, and muscle metaboreflex. *Am J Physiol Heart Circ Physiol* **309,** H381-392.

Mitchell JH. (1990). J.B. Wolffe memorial lecture. Neural control of the circulation during exercise. *Med Sci Sports Exerc* **22,** 141-154.

Murphy MN, Mizuno M, Downey RM, Squiers JJ, Squiers KE & Smith SA. (2013). Neuronal nitric oxide synthase expression is lower in areas of the nucleus tractus solitarius excited by skeletal muscle reflexes in hypertensive rats. *Am J Physiol Heart Circ Physiol* **304,** H1547-1557.

Norton KH, Boushel R, Strange S, Saltin B & Raven PB. (1999a). Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *J Appl Physiol* **87,** 332-338.

Norton KH, Boushel R, Strange S, Saltin B & Raven PB. (1999b). Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *J Appl Physiol (1985)* **87,** 332-338.

O'Neill CA, Stebbins CL, Bonigut S, Halliwell B & Longhurst JC. (1996). Production of hydroxyl radicals in contracting skeletal muscle of cats. *J Appl Physiol (1985)* **81,** 1197-1206.

Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, A OY & Raven PB. (2006). Cardiopulmonary baroreflex is reset during dynamic exercise. *J Appl Physiol (1985)* **100,** 51-59.

Ogoh S, Fadel PJ, Nissen P, Jans O, Selmer C, Secher NH & Raven PB. (2003). Baroreflex-mediated changes in cardiac output and vascular conductance in response to alterations in carotid sinus pressure during exercise in humans. *J Physiol* **550,** 317-324.

Ogoh S, Fisher JP, Dawson EA, White MJ, Secher NH & Raven PB. (2005). Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. *The Journal of physiology* **566,** 599-611.

Ogoh S, Fisher JP, Fadel PJ & Raven PB. (2007). Increases in central blood volume modulate carotid baroreflex resetting during dynamic exercise in humans. *J Physiol* **581,** 405-418.

Osborn JW, Fink GD, Sved AF, Toney GM & Raizada MK. (2007). Circulating angiotensin II and dietary salt: converging signals for neurogenic hypertension. *Current hypertension reports* **9,** 228-235.

Paintal AS. (1973). Vagal sensory receptors and their reflex effects. *Physiol Rev* **53,** 159-227.

Patel KP, Li YF & Hirooka Y. (2001). Role of nitric oxide in central sympathetic outflow. *Exp Biol Med (Maywood)* **226,** 814-824.

Paton JF & Waki H. (2009). Is neurogenic hypertension related to vascular inflammation of the brainstem? *Neuroscience and biobehavioral reviews* **33,** 89-94.

Pawelczyk JA & Raven PB. (1989). Reductions in central venous pressure improve carotid baroreflex responses in conscious men. *Am J Physiol* **257,** H1389-1395.

Potts JT, Shi XR & Raven PB. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *The American journal of physiology* **265,** H1928-1938.

Powers SK, Duarte J, Kavazis AN & Talbert EE. (2010). Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp Physiol* **95,** 1-9.

Powers SK, Radak Z & Ji LL. (2016). Exercise-induced oxidative stress: past, present and future. *J Physiol*.

Prabhakar NR, Dick TE, Nanduri J & Kumar GK. (2007). Systemic, cellular and molecular analysis of chemoreflex-mediated sympathoexcitation by chronic intermittent hypoxia. *Exp Physiol* **92,** 39-44.

Prast H, Dietl H & Philippu A. (1992). Pulsatile release of histamine in the hypothalamus of conscious rats. *J Auton Nerv Syst* **39,** 105-110.

Prast H & Philippu A. (1992a). Nitric oxide releases acetylcholine in the basal forebrain. *Eur J Pharmacol* **216,** 139-140.

Prast H & Philippu A. (1992b). Release of endogenous acetylcholine in the hypothalamus of conscious rats. *Naunyn Schmiedebergs Arch Pharmacol* **346,** 1-3.

Prast H & Philippu A. (2001). Nitric oxide as modulator of neuronal function. *Prog Neurobiol* **64,** 51-68.

Querry RG, Smith SA, Stromstad M, Ide K, Raven PB & Secher NH. (2001a). Neural blockade during exercise augments central command's contribution to carotid baroreflex resetting. *Am J Physiol Heart Circ Physiol* **280,** H1635-1644.

Querry RG, Smith SA, Stromstad M, Ide K, Secher NH & Raven PB. (2001b). Anatomical and functional characteristics of carotid sinus stimulation in humans. *Am J Physiol Heart Circ Physiol* **280,** H2390-2398.

Radak Z, Pucsok J, Mecseki S, Csont T & Ferdinandy P. (1999). Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. *Free Radic Biol Med* **26,** 1059-1063.

Raven PB. (2012). Neural control of the circulation: exercise. *Exp Physiol* **97,** 10-13.

Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L & West MS. (1992a). Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol (1985)* **73,** 1797-1804.

Reid MB, Shoji T, Moody MR & Entman ML. (1992b). Reactive oxygen in skeletal muscle. II. Extracellular release of free radicals. *J Appl Physiol (1985)* **73,** 1805-1809.

Rowell LB. (1993). *Human cardiovascular control*. Oxford University Press, New York ; Oxford.

Rowell LB & O'Leary DS. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J Appl Physiol (1985)* **69,** 407-418.

Sanderford MG & Bishop VS. (2002). Central mechanisms of acute ANG II modulation of arterial baroreflex control of renal sympathetic nerve activity. *American journal of physiology Heart and circulatory physiology* **282,** H1592-1602.

Sartori C, Lepori M & Scherrer U. (2005). Interaction between nitric oxide and the cholinergic and sympathetic nervous system in cardiovascular control in humans. *Pharmacol Ther* **106,** 209-220.

Schultz HD. (2009). Nitric oxide regulation of autonomic function in heart failure. *Curr Heart Fail Rep* **6,** 71-80.

Shim CY, Ha JW, Park S, Choi EY, Choi D, Rim SJ & Chung N. (2008). Exaggerated blood pressure response to exercise is associated with augmented rise of angiotensin II during exercise. *Journal of the American College of Cardiology* **52,** 287-292.

Smith SA, Mitchell JH & Li J. (2005). Independent modification of baroreceptor and exercise pressor reflex function by nitric oxide in nucleus tractus solitarius. *Am J Physiol Heart Circ Physiol* **288,** H2068-2076.

Stocker SD & Toney GM. (2005). Median preoptic neurones projecting to the hypothalamic paraventricular nucleus respond to osmotic, circulating Ang II and baroreceptor input in the rat. *The Journal of physiology* **568,** 599-615.

Tan PS, Killinger S, Horiuchi J & Dampney RA. (2007). Baroreceptor reflex modulation by circulating angiotensin II is mediated by AT1 receptors in the nucleus tractus solitarius. *American journal of physiology Regulatory, integrative and comparative physiology* **293,** R2267-2278.

Vallbo AB & Hagbarth KE. (1968). Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Exp Neurol* **21,** 270-289.

Vallbo AB, Hagbarth KE, Torebjork HE & Wallin BG. (1979). Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* **59,** 919-957.

Villapol S & Saavedra JM. (2015). Neuroprotective effects of angiotensin receptor blockers. *Am J Hypertens* **28,** 289-299.

Volianitis S, Yoshiga CC, Vogelsang T & Secher NH. (2004). Arterial blood pressure and carotid baroreflex function during arm and combined arm and leg exercise in humans. *Acta Physiol Scand* **181,** 289-295.

Waki H, Gouraud SS, Maeda M & Paton JF. (2008). Specific inflammatory condition in nucleus tractus solitarii of the SHR: novel insight for neurogenic hypertension? *Autonomic neuroscience : basic & clinical* **142,** 25-31.

Waki H, Gouraud SS, Maeda M, Raizada MK & Paton JF. (2011). Contributions of vascular inflammation in the brainstem for neurogenic hypertension. *Respiratory physiology & neurobiology* **178,** 422-428.

Wallin BG, Burke D & Gandevia S. (1994). Coupling between variations in strength and baroreflex latency of sympathetic discharges in human muscle nerves. *J Physiol* **474,** 331-338.

Wang S, Paton JF & Kasparov S. (2007). Differential sensitivity of excitatory and inhibitory synaptic transmission to modulation by nitric oxide in rat nucleus tractus solitarii. *Exp Physiol* **92,** 371-382.

Yang W, Oskin O & Krukoff TL. (1999). Immune stress activates putative nitric oxide-producing neurons in rat brain: cumulative effects with restraint. *J Comp Neurol* **405,** 380-387.

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR & Davisson RL. (2002). Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* **91,** 1038-1045.

Zimmerman MC, Lazartigues E, Sharma RV & Davisson RL. (2004). Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res* **95,** 210-216.

Zucker IH. (2006). Novel mechanisms of sympathetic regulation in chronic heart failure. *Hypertension* **48,** 1005-1011.

Zucker IH, Wang W, Pliquett RU, Liu JL & Patel KP. (2001). The regulation of sympathetic outflow in heart failure. The roles of angiotensin II, nitric oxide, and exercise training. *Ann N Y Acad Sci* **940,** 431-443.

CHAPTER II

Baroreflex control of arterial blood pressure during dynamic exercise: A free radical mechanism of arterial baroreflex resetting

Gilbert Moralez[1], Noah P. Jouett[1], Daniel W. White[2], Jun Tian[3], Matthew C.Zimmerman[3] and Peter B. Raven[1]

[1] *Institute for Cardiovascular and Metabolic Disease, University of North Texas Health Science Center, Fort Worth, TX*

[2] *School of Arts & Sciences, University of Houston-Victoria, Victoria, TX*

[3]*Department of Cellular and Integrative Physiology, University of Nebraska Medical Ctr., Omaha, NE*

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Key points

- The operating point (OP) pressure of the arterial baroreflex (ABR) is reset upward and rightward in relation to progressive increases in exercise intensity.
- Resetting of the arterial baroreflex and hence control of sympathetic outflow during exercise may be mediated by free radicals.
- We demonstrate that oral ingestion of a centrally and peripherally acting antioxidant cocktail resulted in a: i) marked reduction in plasma superoxide concentrations; ii) reduction in muscle sympathetic nerve activity (MSNA); iii) downward and leftward resetting of the OP pressure of the ABR at rest and during exercise.
- The results identify that free radicals play a pivotal role in the exercise related resetting of the ABR control of blood pressure and MSNA in humans and may shed light on the pathophysiology of exercise induced hypertension.

ABSTRACT

With progressive increases in dynamic exercise intensity, resetting of the arterial baroreflex (ABR) operating point (OP) pressure is essential to enable the appropriate delivery of oxygen and nutrients to working muscles. However, the molecular signaling mechanisms for exercise-induced ABR-OP resetting in humans have not been determined. This investigation tested the hypothesis that an oral antioxidant cocktail (AOX) would attenuate the exercise induced resetting of the ABR-OP pressure, and therefore, implicate a free radical (FR) signaling mechanism in ABR resetting. Twelve healthy subjects performed 70° back supported semi-recumbent dynamic leg cycling exercise 90 min after ingesting an AOX that has been shown to cross the blood brain barrier (BBB) or placebo (PL). Muscle sympathetic nerve activity (MSNA), mean arterial pressure (MAP), superoxide-dependent electron spin resonance (ESR) spectroscopy were measured and ABR function curves modeled at rest and during cycling exercise at heart rates (HR) of 120 bpm (EX120) and 150 bpm (EX150). The AOX reduced: i) ESR spectra amplitudes; and ii) MSNA burst frequency at rest and during both EX120 and EX150 compared to PL ($P \leq 0.05$). The AOX also caused marked decrease in MAP at rest and at both exercise intensities ($P \leq 0.05$). Furthermore, AOX decreased the ABR-MAP response range, operating point and saturation pressures at rest and during both exercise intensities. We conclude that the AOX scavenged the exercise related free radicals that subsequently resulted in a reduction of central MSNA outflow.

INTRODUCTION

At rest an increase in arterial blood pressure (ABP) activates the arterial baroreceptors located within the carotid sinus and the aortic arch resulting in a centrally mediated reflex increase in parasympathetic nerve activity and a decrease in sympathetic nerve activity (SNA) to elicit bradycardia and reduce ABP (Eckberg *et al.*, 1992). In contrast, during dynamic exercise this negative feedback control system is altered, allowing a positive chronotropic effect on heart rate and an simultaneous increase in ABP (Sagawa, 1983). Melcher and Donald (1981) developed an isolated carotid baroreceptor model in exercising canines and established that the arterial baroreflex (ABR) stimulus-response function curve was reset rightward and upward during treadmill running exercise without any change in arterial baroreflex sensitivity. Work in humans confirmed Melcher & Donald's findings that the operating point (OP) pressure of the ABR function curve is reset upwards and rightwards in direct relation to the increase in dynamic exercise intensity (Potts *et al.*, 1993; Papelier *et al.*, 1994; Norton *et al.*, 1999a; Fadel & Raven, 2012).

Activation of central command (CC) and/or the exercise pressor reflex (EPR) are essential for resetting of the OP pressure of the ABR in direct relation to progressive increases in exercise intensity (Potts *et al.*, 1993; Bishop, 1994; Norton *et al.*, 1999a; McIlveen *et al.*, 2001; Williamson *et al.*, 2006; Fadel & Raven, 2012). When subjects exercise without input from CC and the EPR, the OP pressure does not increase above the resting ABP (Strange *et al.*, 1993). However, the molecular signaling mechanisms that are generated from activation of the EPR and CC that cause exercise induced ABR resetting remain to be elucidated.

A likely signaling candidate is the increased production of free radicals (FR) that occurs during dynamic exercise. Production of centrally and peripherally generated free radicals is directly proportional to exercise intensity (Liu *et al.*, 2000b; Bailey *et al.*, 2004; Powers *et al.*, 2010; Bailey *et al.*, 2011; Powers *et al.*, 2016). Increased production of FR, particularly superoxide, is a result of an increased electron transport chain flux associated with increased metabolism in the brain and in contracting skeletal muscles (Bailey *et al.*,2011; Powers *et al*.,2016) . Animal investigations identify that FRs scavenge nitric oxide (NO) within the nucleus tractus solitarius (NTS) and rostral ventral lateral medulla (RVLM) and thus allows for an increased central SNA outflow and an increase in ABP (Aslan *et al.*, 2001; Waki *et al.*, 2008; Schultz, 2009; Fisher & Fadel, 2010). Furthermore, in animal models of neurogenic hypertension, there is excessive production of FRs within the NTS and RVLM, which results in a pathophysiological increase in central SNA outflow and hypertension (Campese *et al.*, 2004; Waki *et al.*, 2010). We contend that the generation of FRs, in part, cause the upward and rightward resetting of the OP-pressure of the arterial baroreflex during dynamic exercise (Melcher & Donald, 1981; Potts *et al.*, 1993; Norton *et al.*, 1999a; Fadel & Raven, 2012; Michelini *et al.*, 2015) and this mechanism to enables an increase in central SNA outflow and ABP. Since both physiological and pathophysiological increases in central SNA outflow are associated with an increase in free radical production (Liu *et al.*, 2000a; Bailey *et al.*, 2004; Fisher & Fadel, 2010; Powers *et al.*, 2016), we tested the hypothesis that attenuating the central and peripheral production of free radicals using a centrally and peripherally acting antioxidant cocktail (AOX) of Co-enzyme Q and vitamin E and C would decrease MSNA burst frequency and incidence as well as decrease ABR-OP pressures at rest and during exercise. The purpose of

the study was to determine whether FR formation during exercise is necessary to enable exercise intensity related increases in central SNA outflow and ABR resetting.

METHODS

***Subjects*:** Three females (age 25 ± 5 yrs., height 164 ± 12 cm; weight 64 ± 8 kg) and nine males (age 25 ± 1 yr., height 178 ± 6 cm; weight 78 ± 3 kg) voluntarily participated in this study. The subjects were deemed healthy via General Health Questionnaire and physical examination and received a resting seated and standing 12-lead electrocardiographic examination prior to being accepted as participants in the study. Individuals with documented cardiovascular, renal, respiratory, metabolic, neuromuscular or mental disorders, and/or receiving prescribed medication that affects cardiopulmonary function, blood pressure regulation, or oxidative/ inflammatory processes were excluded from participation. Written informed consent was obtained from each of the subjects. Subjects were asked to abstain from caffeine, exercise, alcohol, and foods high in antioxidants (e.g. berries, beans, orange juice) 24hrs prior to all visits to the laboratory. Subjects taking multivitamins regularly were instructed to refrain from ingesting their tablets for at least 15 days before each experimental session. Due to the potential effect of estrogen and progesterone on autonomic neural regulation, female subjects not on hormonal contraception were tested in the early follicular (low hormone) phase of their menstrual cycle. Females taking hormonal contraception were tested during the placebo phase of their contraception cycle. The study conformed to guidelines set forth by the Declaration of Helsinki and was approved (IRB # 2014-089) by the Institutional Review Board for the Protection of Human Subjects in Research at the University of North Texas Health Science Center.

Experimental protocol: The subjects visited the laboratory on three occasions: once for orientation and the other two days for conducting the experimental protocols in which subjects ingested AOX or PL in a randomized cross-over design. Experimental protocols were separated by at least one week during which the subjects were instructed to maintain their normal exercise and diet regimen.

The subjects performed a graded exercise stress test (GXT) to determine their exercise tolerance and work-load for steady-state exercise at heart rates (HR) of 120 and 150 beats/min considered to represent heavy and very heavy work, respectively (Åstrand & Rodahl, 1986). The subjects were also familiarized with wearing the neck collar (for the assessment of ABR function- details below) and underwent 2-3 trials of neck pressure (NP) and neck suction (NS) at rest and during mild exercise.

The antioxidant cocktail (AOX) was based on the work of Lee *et al.*, (2009) who observed that their antioxidant formulation mitigated intermittent hypoxia induced ventilation adaptations in humans, which in animal models is demonstrated to be associated with oxidative stress (Prabhakar et al., 2007).

We compared the efficacy of this AOX to reduce exercise induced oxidative stress against a glucose (< 0.2 mg) placebo. The AOX consisted of 150 IU of Vitamin E (α-tocopheryl), 100 mg Co-enzyme Q10 (Ubiquinone USP Grade, Qunol™, Ultra CoQ10, Fairfield, NJ 07004), and 2 g Vitamin C (Ascorbic Acid; AA). The hydrophilic ascorbic acid scavenges FR in the plasma and cytosol of the peripheral (i.e. not central nervous system) compartment; CoQ10 and Vitamin E are lipophilic antioxidants that reduce oxidative stress both peripherally and centrally (i.e. crossing the blood brain barrier; BBB). Furthermore, the hydrophilic AA and lipophilic Vitamin E synergize their antioxidant effects between the cytosol and cell membrane,

respectively (Chan, 1993). On each experimental day the subjects, ingested either the glucose placebo or the AOX (Lee et al., 2009) in capsule form approximately 90 min before the exercise protocol started; subjects were blinded to the intervention.

Instrumentation and exercise protocol. Once subjects successfully completed the orientation day they were invited to participate in the experimental protocol performed at the same time of day for each subject. After ingestion of either the PL or AOX capsules the subjects were instrumented with a 3-lead ECG (Hewlett-Packard, Inc.) to monitor heart rate and finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) to monitor beat-to-beat ABP. Multiunit muscle sympathetic nerve activity (MSNA) was recorded from the radial nerve at the spiral groove using ultrasound-guided (Curry & Charkoudian, 2011) standardized techniques (Hagbarth & Vallbo, 1968; Wallin et al., 1994; Vallbo et al., 2004). Additionally, a catheter was inserted into an antecubital fossa vein to withdraw 5 mL samples of blood for measurement of peripheral superoxide concentrations, as a measure of FR productions.

The subjects were positioned within a customized box enclosing a stationary cycle ergometer in a 70^0 back supported semi-recumbent position with the legs extended parallel with the floor and feet resting on the pedals. The subjects rested in the semi-recumbent position during instrumentation (20 min) for a minimum 10-min or more without disturbance to establish pre-exercise baseline (BL) values and a blood sample was obtained. Subsequently, pedaling exercise was performed on a computer-controlled magnetically braked ergometer (Intellifit, Houston, Texas). After attaining steady-state circulatory responses (after ~ 5 min at each workload), exercise data were collected for 5 min. Upon completion of the subject's baseline recording a blood sample was obtained and then the carotid baroreflex control of mean arterial

pressure (MAP) was assessed as describe by (Potts *et al.* (1993); Norton *et al.* (1999a)). The two exercise workloads were performed continuously for approximately 20 min each.

ABR function: Manipulation of the carotid baroreflex was performed by a customized computer controlled variable pressure neck collar fitted onto the anterior 2/3 of the subject's neck (Pawelczyk & Raven, 1989; Potts *et al.*, 1993). The collar provides an airtight chamber which encompasses the location of the carotid sinus, which was verified by ultrasound imaging. The computer software initiates a positive (neck pressure, NP) or negative (neck suction, NS) pressure pulse for 5 sec. Pressures were randomly applied between +40 and -60 Torr in 20 Torr increments. Within the collar 89% of the NP and 83% of the NS, respectively, are transmitted to the carotid baroreceptors and are sensed as changes in arterial transmural pressure (Potts *et al.*, 1993). The NP simulates hypotension and NS simulates hypertension as sensed by the carotid baroreceptors. Beat-to-beat measurements of MAP were analyzed to determine the responses to NP/NS stimulation. Carotid baroreflex (CBR) function curves were constructed using the inverse logistic function of Kent *et al.* (1972) without correcting for degree of transmission. Threshold and saturation calculations were determined by applying a correction of the exponential developed by McDowall and Dampney (2006) to the Kent logistic function algorithm.

Measurement of Free Radicals: Electron spin resonance (ESR) spectroscopy was utilized to measure peripheral free radicals activity, in particular superoxide ($[O_2\cdot^-]$), in whole-blood samples. Triplicate 200 μL samples for each time point (baseline, EX120, and EX150) were incubated for 15 min in a buffer solution containing: 3.5 mM deferoxamine methanesulfonate salt (DF), 9.08 mM diethyldithiocarbamic acid sodium (DETC), and Krebs-HEPES buffer (Noxygen Science Transfer & Diagnostics GmbH, Elzach, Germany) containing methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH) as spin probe, which preferentially

detects $O_2 \cdot^-$ (Deo *et al.*, 2012; Vianna *et al.*, 2015; Jouett *et al.*, 2016). Triplicate 50 µl samples of whole blood for each time point in triplicate were then loaded into a 1-cc syringe and flash frozen using liquid nitrogen between buffer solutions to form a continuous frozen plug. Samples were then stored at −80°C and shipped to the University of Nebraska Medical Center's ESR Spectroscopy Core for analysis (Deo *et al.*, 2012; Vianna *et al.*, 2015). ESR amplitude was measured using a Bruker e-Scan ESR Spectrometer and was averaged for all triplicate data to generate an individual average for each time point.

Data acquisition and analyses: Electronically recorded data were sampled at 500 Hz and recorded to a computer via data acquisition software (WINDAQ, Dataq Instruments, Akron, OH). The data were then analyzed using a biomedical analysis software program (WinCPRS, Absolute Aliens, Turku, Finland). Data from the last 2 min of the 10 min BL were analyzed. Exercise data were analyzed in 2 min epochs during EX120 and during EX150 after 3 min of steady-state exercise. R-waves generated from the ECG were detected and marked at their occurrence in time. Systolic and diastolic pressures (SAP, DAP) were marked from the arterial pressure waveforms and MAP was calculated as the area under the arterial pressure waveforms via the WinCPRS software. Beat-to-beat stroke volume (SV) and cardiac output (Q) were estimated using the pulse contour method (Jansen et al., 1990). MSNA signals were band-pass filtered (100–2000 Hz), and integrated (time constant, 0.1 s) to obtain mean voltage neurograms. Neurograms were subsequently imported into WinCPRS that has the capability of detecting bursts of MSNA. The software detects a burst of MSNA based on two primary criteria: i) pulse synchronous spontaneous bursts with signal-to-noise ratios of approximately 3 :1; and ii) reflex latencies from preceding R-waves of approximately 0.9 s (Wallin et al., 1994; Cooke et al., 2009). The same experienced microneurographer (GM) manually checked the computerized

burst detection results. MSNA was expressed as bursts per minute, i.e. burst frequency (BF) and as bursts per 100 heart beats, i.e. burst incidence (BI). Due to the baseline fluctuations of the neurograms that occur during heavy intensity exercise, the amplitude and area of sympathetic bursts were not expressed. Furthermore, analyses of MSNA burst amplitude and area (e.g. MSNA total activity) are further unwarranted because MSNA burst amplitude and area are related to the position of the electrode in relation to the sympathetic efferent fascicle and it could not be verified that the electrode was placed in the identical location on the different experimental days (White *et al.*, 2015).

ABR stimulus-response function curves: Arterial baroreflex stimulus-response curves for MAP were modeled for each subject to the four-parameter logistic function described by Kent *et al.*, (1972):

$$MAP = A_1 \{1 + \exp [A_2 (ECSP - A_3)] \}^{-1} + A_4$$

where MAP is the dependent variable, ECSP is the estimated carotid sinus pressure, A_1 is the range (i.e. maximum – minimum), A_2 is the gain coefficient (average of the slope of the function), A_3 is the value at the centering point (i.e. point of maximum gain), and A_4 is the minimum response of MAP. Furthermore, the carotid sinus threshold pressures (the point where no further increase in MAP was elicited by reductions in ECSP; THR) and saturation (SAT) pressures (the point where no further decrease in MAP was elicited by an increase in ECSP) were calculated using the following equations:

Saturation: $A_3+2.944/A_2$

Threshold: $A_3-2.944/A_2$

A_1-A_4 were defined as previously describe (Potts *et al.*, 1993; McDowall & Dampney, 2006). The OP pressure of each curve was defined as a stimulus ECSP and averaged over the respective time points (rest, EX120, EX150) after circulatory responses obtained steady-state. Hence, for each subject and condition, the OP, SAT and THR pressures were calculated and compared between and across subjects.

Statistical analyses: Based on changes in ABP expected during exercise, and using a Student's t-test based power calculation, we estimated that a sample size of 12 subjects would be adequate to test our hypothesis with an $\alpha = 0.05$ and $1-\beta = 0.8$. Therefore, considering the potential subject withdrawal and MSNA recording success rate ~30%, we targeted, recruited and screened 32 subjects.

All data were analyzed with (SigmaPlot software, Systat Software Inc., California, USA). We tested for differences among the mean values of interest between baseline and exercise for each experimental condition (AOX vs PL) using two-factor repeated measures ANOVA. For assessment of differences among the mean values of interest between baseline and exercise, a two (treatment; PL and AOX) by three (time; baseline, EX120 and EX150) factorial ANOVA for repeated measures was implemented. A Student-Newman-Keuls post hoc test was conducted to determine differences between groups. Data are presented as means ± SE and statistical significance was accepted as P values < 0.05

RESULTS

Six subjects withdrew after the initial GXT session, eleven subjects after the first experimental session, and three subjects withdrew during the second experimental session. Therefore, 12 subjects completed both of the experimental steady-state protocols and comprised

the studied group. Acceptable repeated measures of plasma superoxide concentrations, MSNA and records of ABP were obtained across both experimental days from only eight of the 12 subjects. Acceptable carotid baroreflex stimulus-response curve repeated measures recordings of ABP were obtained from 8 of the 12 subjects, four of whom were different individuals than the 8 subjects who had successful repeated MSNA recordings under the two exercise conditions. Due to the technical challenge of obtaining reliable recordings of MSNA during heavy and/or very heavy intensity exercise while performing the carotid baroreflex function curve protocol, the ABR-MSNA responses could not be modeled by the Kent logistic model. Furthermore, workload was unchanged between conditions within each subject (female subjects: EX120, 50 ± 2 W; EX150, 83 ± 18 W; male subjects: EX120, 91 ± 12 W; EX150, 150 ± 16 W).

Arterial BP responses to antioxidant cocktail during rest and exercise: Oral ingestion of the AOX resulted in a marked reduction in plasma superoxide concentrations ($[O_2 \cdot -]$) (N=8, $P < 0.05$) (figure 1A & 1B). The $[O_2 \cdot -]$ increased from baseline to EX120 both in PL and AOX conditions ($P \leq 0.05$) and remained elevated throughout EX150 with no further increases in $[O_2 \cdot -]$ with the increased workload ($P \geq 0.21$, Fig 1). Furthermore, EX150 $[O_2 \cdot -]$ were statistically indistinguishable compared to baseline for both treatment conditions (PL, P =0.09; CT, P =0.35). Additionally, oral ingestion of the AOX reduced $[O_2 \cdot -]$ during rest and both exercise workloads compared to PL (figure 1A ($P \leq 0.05$, Fig. 1).

SAP and MAP were reduced with AOX ($P < 0.05$) with a trending reduction in DAP (P = 0.06; n=12) resulting in a lower resting MAP at baseline (P =0.01; n=12). A summary of the hemodynamic variables is shown in table 1.The AOX reduced the MAP response to exercise during both workloads ($P \leq 0.05$, Fig. 2). The reduction in MAP can be attributed to the change in SAP with no alteration in DAP during exercise ($P \geq 0.20$; Table 1). Figures 2A, B & C

identify progressive increases in MSNA and blood pressure from rest to exercise during both workloads and conditions (N=8). In addition, leg cycling exercise elicited significant increases in HR, SV and Q under both conditions compared to baseline and progressively increased from EX120 to EX150 with the exception of SV (PL, P = 0.1; AOX, P =0.07). Notably, the exercise-induced increases in HR were significantly attenuated with AOX during both workloads ($P \leq 0.02$).

MSNA responses to dynamic exercise with and without antioxidant cocktail:

Fig. 3 is a comparison of a representative subject's MSNA and ABP recordings comparing baseline, EX120 and EX150. Compared to placebo, the AOX burst frequency (BF) of MSNA at rest was decreased 4 bursts/min, 8 bursts/min at EX120 and 12 bursts/min at EX150 (P =0.02; n=8 (figure 2A)). The repeated measures ANOVA identified an interaction of MSNA BF with PL and AOX at different exercise conditions of EX120 and Ex150 ($P \leq 0.05$). The reduction in superoxide concentrations and MSNA are reflected in a notable reduction in the MAP in AOX condition at rest, EX120 and EX150 compared with PL. However, there was no difference in MAP between EX120 and EX150 within the same condition (Fig 2C).

Dynamic leg cycling exercise provoked significant increases in MSNA (BF) from rest to EX120 under both conditions (figure 2A) ($P < 0.01$)). In addition, unlike the [O2·−], MSNA-BF at EX150 was significantly increased 12 bursts/min above the BF in the EX120 in PL but only increased 8 burst/min in AOX conditions ($P < 0.01$). However, the MSNA-BF increase between baseline and EX120 was 3 burst/min less in AOX compared to PL ($P < 0.01$) and the MSNA-BF increase between EX120 and EX150 was significantly less with AOX 5 bursts/min when compared with placebo ($P < 0.01$). Normalization of MSNA to bursts/100 heart beats (BI)

(White *et al.*, 2015) identified that the AOX reduced BI at rest insignificantly 2 to 3 bursts/100 HB and during EX 120 and EX150 the reduction in BI was significantly reduced by 5 to 6 bursts/100HB ($P \leq 0.05$ (figure 2B)).

Arterial baroreflex control of mean arterial pressure with and without antioxidant cocktail:

The stimulus-response relationship for the arterial baroreflex control of MAP at rest and during both workloads of steady-state dynamic leg cycling exercise is presented in figures 4A & B. For a clearer interpretation of the data each stimulus response curve was plotted individually on one graph for each workload with baseline, i.e. the ABR-MAP function curve at rest. Furthermore, logistic parameters describing the ABR-MAP relationship are shown in table 2.

Oral ingestion of AOX decreased the ABR-MAP response range during baseline and throughout dynamic exercise ($P \leq 0.05$). However, exercise did not alter the ABR-MAP response range under both conditions when compared to baseline ($P \geq 0.10$). In addition, the Gmax at the centering point (CP pressure = where an equal pressor and depressor response occurs) of the ABR-MAP stimulus response relationship was similar at rest and during both exercise workloads and was not affected by AOX throughout the protocol ($P \geq 0.50$; table 2). On the other hand, arterial pressures at the OP,THR and SAT pressures were significantly increased from baseline to EX120 (i.e. resetting) with no further changes in the OP pressure during EX150 under both conditions. Moreover, AOX significantly decreased the OP pressures at BL, EX120 and EX150 indicating that FRs are involved in establishing the OP pressure of the ABR at rest, and dynamic exercise figure 4A & 4B.

DISCUSSION

The present study demonstrated that free radicals, particularly superoxide, (Figs. 1A & B) are involved in establishing the resting and exercise intensity related OP-pressures of the arterial baroreflex (Figs. 2A, 2B & 2C). These findings support our hypothesis that free radicals generated during exercise in an intensity-dependent fashion are involved in the: (i) increase in MSNA observed with dynamic exercise; and (ii) increase in arterial pressure which accompanied the resetting of the ABR-OP pressure. Indeed, the dynamic exercise induced increase in the OP pressure, i.e. the exercise induced "resetting" of the OP-pressure of the ABR was attenuated by AOX compared to PL.

Baroreflex Resetting: Rowell and O'Leary (1990) proposed a hypothetical model of ABR "resetting" during exercise. Their model proposed that at exercise onset and subsequent increases in exercise intensity CC resets the OP pressure of the arterial baroreflex stimulus-response curve relationship laterally to higher OP pressures by acting centrally on the neuron pool receiving the baroreceptor afferents; while at the same time a vertical shift in the baroreflex function curves was affected by the exercise pressor reflex (EPR), which increased sympathetic nerve activity without changing the OP pressure because the stimulus acts only on the efferent arm of the reflex and not on the central neurons controlling the reflex. The resulting vector of the horizontal and vertical shifts predicted by the modeled increases in sympathetic activity was proposed to result in an upward and rightward "resetting" of the OP pressure around which the ABR operated. The hypothetical model poposed by Rowell and O'Leary was subsequently confirmed in dynamically exercising human subjects performing progressive increases in exercise intensity using a customized NP/NS system (Pawelczyk & Raven, 1989) and modeled ABR-HR and -MAP responses (Potts *et al.*, 1993; Norton *et al.*, 1999a; Fadel & Raven, 2012).

Numerous reviews of both animal and human investigations have identified that selective activation or blockade of CC (Williamson, 2010) and/or the EPR (Raven, 2012; Fisher *et al.*, 2015; Michelini *et al.*, 2015) were required to affect "positive" or "negative" resetting, respectively, of the ABR-OP pressure during exercise. Indeed, in a unique experimental investigation (Strange *et al.*, 1993) in which human subjects performed: i) voluntary dynamic exercise (a condition where both CC and EPR provided input to the NTS) arterial blood pressure increased; ii) where electrical stimulation performed the dynamic leg exercise (i.e. a condition of exercise performed with no CC input to the NTS) arterial blood pressure was increased; iii) where electrical stimulation elicited dynamic leg exercise after induction of epidural anesthesia (i.e. a condition of dynamic exercise with no CC and no EPR input to the NTS) the blood pressure was unchanged from rest. In this investigation, the effect of ingesting the oral AOX on the ABR-MAP stimulus response curves, modeled from the NP/NS invoked ABP responses at rest and during exercise, manifested as bidirectional relocation downward on the sympathetic arm and a leftward shift to lower OP pressures at BL and during dynamic cycling exercise. However, the magnitude of the reduction in the OP - MAP tended to be greater from rest to EX120 than between rest and EX120 to EX150 ($P>0.08$), see Figs. 4A & B, despite the presence of a greater reduction in MSNA – BF between rest and EX120 and EX150 (Fig 2A). One possible explanation for this apparent discrepancy between the increases in exercise-intensity related MSNA-BF and increases in MAP at EX120 to EX150 is the presence of the cellular mechanisms involved in vascular transduction mechanisms of sympatholysis (Remensnyder *et al.*, 1962) and cardiovascular drift (Ekelund, 1967; Norton *et al.*, 1999b).

Free Radical Mediated Increases in Sympathetic Nerve Activity during Dynamic Exercise: Superoxide has been found to scavenge nitric oxide (NO) within the NTS and RVLM enabling increased central SNA outflow (Aslan *et al.*, 2001; Waki *et al.*, 2008; Schultz, 2009; Fisher & Fadel, 2010). In the present investigation ingestion of the oral AOX manifests as a reduction of the superoxide-dependent ESR spectra amplitude measured in the blood samples obtained at rest and during Ex120 and Ex150. The resonance amplitude is reflective of the plasma superoxide anion concentration, see Figs. 1A & 1B. A novel finding of the present investigation is due to the repeated successful recordings of radial MSNA during rest and EX120 and EX150 during dynamic exercise in 8 subjects. This technological success enabled the completion of a randomized repeated measures design investigation. The progressive increase in the attenuation of the MSNA burst frequency (BF) from rest to Ex120 to Ex150 (see Fig. 2A) and the consequent reduction of the OP - MAP (Fig. 2C and Figs. 4A & 4B) identifies an interaction between the exercise intensity related increase in the AOX scavenging of the central production of superoxide and MSNA-BF.

In our protocol we reasoned that the AOX has minimal peripheral action on the EPR at rest, hence, without the activation of the chemoreflex the AOX resulted in a leftward shift of the baroreflex function curve, as indicated by the reduction in the saturation pressure. In line with Rowell & O'Leary's hypothesis (1990) this shift is most likely a result of decreased central activation. Furthermore, if the AOX was causing a direct effect on the vasculature this would have resulted in a baroreflex mediated increase in SNA outflow and HR. However, in our protocol the HR was unchanged at rest and was significantly decreased during exercise in the AOX condition. The exercise intensity related increases in HR from rest are a result of progressive changes from a vago-sympathetic balance to a sympatho-vagal balance occurring at

HRs of 140beats/min that increases exponentially up to HRmax (White & Raven, 2014). We contend that in our protocol the central action of the AOX attenuated the exercise intensity related increases in HR. Indeed, when given intravenously Campese *et al.* (2004) demonstrated that the antioxidant Tempol exerted a direct vasodilation on the peripheral circulation and caused a reflex activation of renal sympathetic nerve activity, which additionally resulted in an increase in HR. Furthermore, the effects of intravenous tempol on sympathetic outflow were blunted or abolished by sinoaortic denervation. In contrast, the intracerebroventricular infusion of Tempol resulted in a reduction in RSNA which caused a reduction in ABP and HR. The authors concluded that the discrepancy in the results between intracerebroventricular and intravenous infusion of Tempol can best be explained by direct central inhibitory action on central SNA outflow. Therefore, we conclude that the primary mechanism by which AOX results in a reduction in the OP pressure and central MSNA outflow is related to its central scavenging of the superoxide and re-establishing the NO buffering of SNA.

An involvement of AOX blockade of the peripheral arterial chemoreceptors (PAC) in the central reduction of MSNA requires the chronic adaptations of the PACs to pathological conditions, such as the intermittent hypoxia of sleep apnea (Prabhakar *et al.*, 2007) or heart failure (Sala-Mercado *et al.*, 2007). In a recent review Michelini *et al.* (2015) provides evidence that the resetting of the ABR-OP pressure is the pivotal mechanism involved in the required resetting of the ABR during increasing dynamic exercise intensities.

In the present investigation, the AOX reduced the response range (i.e. the sympathetic arm of the ABR function curve that is activated by NP's simulation of hypotension) indicating that AOX attenuated the central SNA responses not only at rest but throughout exercise. The combination of the attenuated central SNA outflow (i.e. the decreased response range) and

reduction of saturation resulted in a downward resetting of the OP pressure at rest. One explanation for the downward resetting of the OP pressure after 30 min of semi-recumbent rest prior to exercise in the AOX condition compared with the PL condition, is that the central neural anticipatory stimulation of SNA associated with CC prior to the onset of exercise (Williamson, 2010) is blunted. Another possible explanation of the AOX reduction in central SNA outflow at rest is that as the subjects are seated in a 70° position for approximately 30 minutes prior to exercise onset, the unloading of the cardiopulmonary baroreceptors reduces the afferent neural inhibition of central SNA outflow at the NTS (Collins & DiCarlo, 1993; DiCarlo *et al.*, 1996), which when AOX is present scavenges the central FRs and reinstates the central NO inhibition of SNA outflow (Campese *et al.*, 2004; Waki *et al.*, 2010).

Perspectives

Our current investigation utilized an integrative approach to identifying the physiological mechanisms for exercise-induced ABR resetting in healthy individuals. The data identify an association between increasing dynamic exercise intensities, free radical production and their involvement in arterial baroreflex resetting and reduced SNA outflow. This investigation not only advances our understanding regarding the physiological mechanisms governing reflex cardiovascular control during exercise in healthy humans but also may provide insight into the patho-physiological mechanisms of impaired autonomic regulation of cardiovascular responses such as exercise induced hypertension (EIHt). EIHt is a clinical disorder that has a 5-year prognosis for humans to develop essential hypertension. EIHt is caused by an impairment of the arterial baroreflex and is defined as a systolic arterial blood pressure (SAP) value exceeding the 90^{th} percentile, equating to a SAP of greater than 210 mmHg for males and greater than 190

mmHg for females. In addition, EIHt has been linked to augmented exercise induced increases in angiotensin II (Ang II) (Shim *et al.*, 2008) . EIHt at moderate exercise intensity is an independent risk factor for cardiovascular events and mortality (Schultz *et al.*, 2013). Furthermore, FRs are produced by: i) metabolic production via the central electron transport chain associated with neural-metabolic processes; and ii) activation of the Renin-Angiotensin system (RAS). Specifically, Ang II stimulation of NAD(P)H oxidase–dependent generation of superoxide peripherally (Griendling *et al.*, 1994) and centrally (Zimmerman *et al.*, 2002; Chan *et al.*, 2005; Zucker, 2006). All of these processes resulting in FR production modify central sympathetic neural outflow (Zimmerman *et al.*, 2004; Campese *et al.*, 2005; Han *et al.*, 2005) . The superoxide radical modifies sympathetic neural outflow by scavenging NO which acts as a sympatho-inhibitory molecule in the CNS thereby enabling an increased central sympathetic neural outflow (Jiang *et al.*, 1996; Aslan *et al.*, 2001; Waki *et al.*, 2008; Schultz, 2009; Fisher & Fadel, 2010). It is well known that exacerbated increases in central oxidative stress are implicated in impaired autonomic regulation of cardiovascular function associated with aging (Monahan *et al.*, 2004), hypertension (Grassi *et al.*, 2004), chronic heart failure (Nightingale *et al.*, 2003), and obstructive sleep apnea (Yamauchi & Kimura, 2008). Furthermore, the Ang II linked FR production is recognized as a major mechanism involved in neurogenic hypertension (Paton & Waki, 2009; Waki *et al.*, 2011; Zubcevic *et al.*, 2011). As such, future studies should consider identifying the central and peripheral roles of Ang II linked free radical production in the exercise induced resetting of the OP pressure of the arterial baroreflex.

Methodological Considerations

Considering that there was a reduction in HR caused by AOX during exercise (Table 1) and reasoning that pulse synchrony remained during increases in exercise intensity, expressing MSNA using BI as an index of increases in sympathetic activity instead of BF results in a misinterpretation of the findings. However, during exercise the intensity related increases in HR from rest are a result of progressive changes from a vago-sympathetic balance to a sympatho-vagal balance and that increases exponentially up to HRmax (White & Raven, 2014). Hence, HR increases related to increases in exercise intensity are reflective of increases in absolute sympathetic activity as identified by BF. In line with the aforementioned BF results, BI MSNA was significantly elevated throughout exercise within the PL condition when compared to the same subjects in the AOX condition (figure 4B ($P \leq 0.03$)).

An unintended consequence of having the subjects perform EX120 and EX150 continuously in series resulted in a progressive downward drift in MAP of 5-7 mmHg. This phenomenon is known as cardiovascular drift (CVD) (Ekelund, 1967)(Norton et al., 1999). However, the BF of MSNA was significantly greater during the EX150 than the EX120 indicating that the cellular mechanisms of functional sympatholysis involved in the CVD also attenuated the MSNA‘s transduction across the peripheral blood vessels, thereby, may reduce the amount of sympathetic vasoconstriction that would be reflected in the exercise induced upward resetting of the OP pressure.

CONCLUSIONS

The question addressed in this investigation was whether or not free radicals being formed during exercise are fundamental to the exercise intensity related ABR resetting. The main finding identifies that indeed free radical production, particularly superoxide, plays a pivotal role in the

exercise related rightward and upward resetting of the arterial baroreflex OP pressure and the reflex control of central sympathetic outflow. Unique features of this investigation include: i) the implementation of radial nerve microneurography during repeated bouts of dynamic leg exercise; and ii) the pharmacological attenuation of FR production, thereby attenuating sympathetic nerve activity in healthy human subjects. From these data, we conclude that the AOX scavenged the exercise related increase in free radical concentration resulting in an increased attenuation of central MSNA outflow during dynamic exercise most likely through a NO mediated mechanism (Campese *et al.*, 2004; Waki *et al.*, 2011).

REFERENCES

Aslan M, Ryan TM, Adler B, Townes TM, Parks DA, Thompson JA, Tousson A, Gladwin MT, Patel RP, Tarpey MM, Batinic-Haberle I, White CR & Freeman BA. (2001). Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Nat Acad Sci USA* **98,** 15215-15220.

Åstrand P-O & Rodahl K. (1986). *Textbook of work physiology : physiological bases of exercise*. McGraw-Hill, New York ; London.

Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, Ogoh S, Ainslie PN, Lucchesi C, Rockenbauer A, Culcasi M & Pietri S. (2011). Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. *Exp Physiol* **96,** 1196-1207.

Bailey DM, Young IS, McEneny J, Lawrenson L, Kim J, Barden J & Richardson RS. (2004). Regulation of free radical outflow from an isolated muscle bed in exercising humans. *Am J Physiol Heart Circ Physiol* **287,** H1689-1699.

Bishop VS. (1994). "Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise". *J Appl Physiol* **77,** 491-492.

Campese VM, Shaohua Y & Huiquin Z. (2005). Oxidative stress mediates angiotensin II-dependent stimulation of sympathetic nerve activity. *Hypertension* **46,** 533-539.

Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z & Chiu J. (2004). Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* **287,** H695-703.

Chan AC. (1993). Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol* **71,** 725-731.

Chan SH, Hsu KS, Huang CC, Wang LL, Ou CC & Chan JY. (2005). NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38 mitogen-activated protein kinase in the rostral ventrolateral medulla. *Circ Res* **97,** 772-780.

Collins HL & DiCarlo SE. (1993). Cardiac afferents attenuate the muscle metaboreflex in the rat. *J Appl Physiol (1985)* **75,** 114-120.

Cooke WH, Rickards CA, Ryan KL, Kuusela TA & Convertino VA. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol* **587,** 4987-4999.

Deo SH, Fisher JP, Vianna LC, Kim A, Chockalingam A, Zimmerman MC, Zucker IH & Fadel PJ. (2012). Statin therapy lowers muscle sympathetic nerve activity and oxidative stress in patients with heart failure. *Am J Physiol Heart Circ Physiol* **303,** H377-385.

DiCarlo SE, Chen CY & Collins HL. (1996). Onset of exercise increases lumbar sympathetic nerve activity in rats. *Med Sci Sports Exerc* **28,** 677-684.

Eckberg DL, Sleight P & Folkow B. (1992). *Human baroreflexes in health and disease*. Clarendon Press, Oxford.

Ekelund LG. (1967). Circulatory and respiratory adaptation during prolonged exercise of moderate intensity in the sitting position. *Acta Physiol Scand* **69,** 327-340.

Fadel PJ & Raven PB. (2012). Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. *Exp Physiol* **97,** 39-50.

Fisher JP & Fadel PJ. (2010). Therapeutic strategies for targeting excessive central sympathetic activation in human hypertension. *Exp Physiol* **95,** 572-580.

Fisher JP, Young CN & Fadel PJ. (2015). Autonomic adjustments to exercise in humans. *Compr Physiol* **5,** 475-512.

Grassi G, Seravalle G, Dell'Oro R, Facchini A, Ilardo V & Mancia G. (2004). Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. *J Hyperten* **22,** 1747-1753.

Griendling KK, Minieri CA, Ollerenshaw JD & Alexander RW. (1994). Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* **74,** 1141-1148.

Hagbarth KE & Vallbo AB. (1968). Discharge characteristics of human muscle afferents during muscle stretch and contraction. *Exp Neurol* **22,** 674-694.

Han Y, Zhang Y, Wang HJ, Gao XY, Wang W & Zhu GQ. (2005). Reactive oxygen species in paraventricular nucleus modulates cardiac sympathetic afferent reflex in rats. *Brain Res* **1058,** 82-90.

Jansen JR, Wesseling KH, Settels JJ & Schreuder JJ. (1990). Continuous cardiac output monitoring by pulse contour during cardiac surgery. *Eur Heart J* **11 Suppl I,** 26-32.

Jiang H, Rummage JA, Stewart CA, Herriott MJ, Kolosova I, Kolosov M & Leu RW. (1996). Evidence for endogenous C1q modulates TNF-alpha receptor synthesis and autocrine binding of TNF-alpha associated with lipid A activation of murine macrophages for nitric oxide production. *Cell Immunol* **170,** 34-40.

Jouett NP, Smith ML, Zimmerman MC & Raven PB. (2016). N-Acetyl Cysteine reduces hyper-acute intermittent hypoxia induced sympathoexcitation in human subjects. *Exp Physiology* **101**, 387–396.

Kent BB, Drane JW, Blumenstein B & Manning JW. (1972). A mathematical model to assess changes in the baroreceptor reflex. *Cardiology* **57,** 295-310.

Lee DS, Badr MS & Mateika JH. (2009). Progressive augmentation and ventilatory long-term facilitation are enhanced in sleep apnoea patients and are mitigated by antioxidant administration. *J Physiol* **587,** 5451-5467.

Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, Brooks GA & Ames BN. (2000a). Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol* **89,** 21-28.

Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, Brooks GA & Ames BN. (2000b). Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol* **89,** 21-28.

McDowall LM & Dampney RA. (2006). Calculation of threshold and saturation points of sigmoidal baroreflex function curves. *Am J Physiol Heart Circ Physiol* **291,** H2003-2007.

McIlveen SA, Hayes SG & Kaufman MP. (2001). Both central command and exercise pressor reflex reset carotid sinus baroreflex. *Am J Physiol Heart Circ Physiol* **280,** H1454-1463.

Melcher A & Donald DE. (1981). Maintained ability of carotid baroreflex to regulate arterial pressure during exercise. *Am J Physiol Heart Circ Physiol* **241,** H838-849.

Michelini LC, O'Leary DS, Raven PB & Nobrega AC. (2015). Neural control of circulation and exercise: a translational approach disclosing interactions between central command, arterial baroreflex, and muscle metaboreflex. *Am J Physiol Heart Circ Physiol* **309,** H381-392.

Monahan KD, Eskurza I & Seals DR. (2004). Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men. *Am J Physiol Heart Circ Physiol* **286,** H2113-2117.

Moralez G, Romero SA, Rickards CA, Ryan KL, Convertino VA & Cooke WH. (2012). Effects of dehydration on cerebrovascular control during standing after heavy resistance exercise. *J Appl Physiol* **112,** 1875-1883.

Nightingale AK, Blackman DJ, Field R, Glover NJ, Pegge N, Mumford C, Schmitt M, Ellis GR, Morris-Thurgood JA & Frenneaux MP. (2003). Role of nitric oxide and oxidative stress in baroreceptor dysfunction in patients with chronic heart failure. *Clin Sci (Lond)* **104,** 529-535.

Norton KH, Boushel R, Strange S, Saltin B & Raven PB. (1999a). Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *J Appl Physiol* **87,** 332-338.

Norton KH, Gallagher KM, Smith SA, Querry RG, Welch-O'Connor RM & Raven PB. (1999b). Carotid baroreflex function during prolonged exercise. *J Appl Physiol* **87,** 339-347.

Papelier Y, Escourrou P, Gauthier JP & Rowell LB. (1994). Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise. *J Appl Physiol* **77,** 502-506.

Paton JF & Waki H. (2009). Is neurogenic hypertension related to vascular inflammation of the brainstem? *Neurosci Biobehav Rev* **33,** 89-94.

Pawelczyk JA & Raven PB. (1989). Reductions in central venous pressure improve carotid baroreflex responses in conscious men. *Am J Physiol* **257,** H1389-1395.

Potts JT, Shi XR & Raven PB. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* **265,** H1928-1938.

Powers SK, Duarte J, Kavazis AN & Talbert EE. (2010). Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp Physiol* **95,** 1-9.

Powers SK, Radak Z & Ji LL. (2016). Exercise-induced oxidative stress: past, present and future. *J Physiol*.

Prabhakar NR, Dick TE, Nanduri J & Kumar GK. (2007). Systemic, cellular and molecular analysis of chemoreflex-mediated sympathoexcitation by chronic intermittent hypoxia. *Exp Physiol* **92,** 39-44.

Raven PB. (2012). Neural control of the circulation: exercise. *Exp Physiol* **97,** 10-13.

Remensnyder JP, Mitchell JH & Sarnoff SJ. (1962). Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* **11,** 370-380.

Rowell LB & O'Leary DS. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J Appl Physiol* **69,** 407-418.

Sagawa K. (1983). Baroreflex Control of Systemic Arterial Pressure and Vascular Bed. *Handbook of Physiology* **Vol.III** Part 2, 453-496

Sala-Mercado JA, Hammond RL, Kim JK, McDonald PJ, Stephenson LW & O'Leary DS. (2007). Heart failure attenuates muscle metaboreflex control of ventricular contractility during dynamic exercise. *Am J Physiol Heart Circ Physiol* **292,** H2159-2166.

Schultz HD. (2009). Nitric oxide regulation of autonomic function in heart failure. *Current heart failure reports* **6,** 71-80.

Schultz MG, Otahal P, Cleland VJ, Blizzard L, Marwick TH & Sharman JE. (2013). Exercise-induced hypertension, cardiovascular events, and mortality in patients undergoing exercise stress testing: a systematic review and meta-analysis. *Am J Hyperten* **26,** 357-366.

Shim CY, Ha JW, Park S, Choi EY, Choi D, Rim SJ & Chung N. (2008). Exaggerated blood pressure response to exercise is associated with augmented rise of angiotensin II during exercise. *J Am Col Cardiol* **52,** 287-292.

Strange S, Secher NH, Pawelczyk JA, Karpakka J, Christensen NJ, Mitchell JH & Saltin B. (1993). Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol* **470,** 693-704.

Vallbo AB, Hagbarth KE & Wallin BG. (2004). Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* **96,** 1262-1269.

Vianna LC, Deo SH, Jensen AK, Holwerda SW, Zimmerman MC & Fadel PJ. (2015). Impaired dynamic cerebral autoregulation at rest and during isometric exercise in type 2 diabetes patients. *Am J Physiol Heart Circ Physiol* **308,** H681-687.

Waki H, Gouraud SS, Maeda M & Paton JF. (2008). Specific inflammatory condition in nucleus tractus solitarii of the SHR: novel insight for neurogenic hypertension? *Autonomic neuroscience : basic & clinical* **142,** 25-31.

Waki H, Gouraud SS, Maeda M & Paton JF. (2010). Evidence of specific inflammatory condition in nucleus tractus solitarii of spontaneously hypertensive rats. *Exp Physiol* **95,** 595-600.

Waki H, Gouraud SS, Maeda M, Raizada MK & Paton JF. (2011). Contributions of vascular inflammation in the brainstem for neurogenic hypertension. *Respir Physiol Neurobiol* **178,** 422-428.

Wallin BG, Burke D & Gandevia S. (1994). Coupling between variations in strength and baroreflex latency of sympathetic discharges in human muscle nerves. *J Physiol* **474,** 331-338.

White DW & Raven PB. (2014). Autonomic neural control of heart rate during dynamic exercise: revisited. *J Physiol* **592,** 2491-2500.

White DW, Shoemaker JK & Raven PB. (2015). Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci*. **193,** 12-21.

Williamson JW. (2010). The relevance of central command for the neural cardiovascular control of exercise. *Exp Physiol* **95,** 1043-1048.

Williamson JW, Fadel PJ & Mitchell JH. (2006). New insights into central cardiovascular control during exercise in humans: a central command update. *Exp Physiol* **91,** 51-58.

Yamauchi M & Kimura H. (2008). Oxidative stress in obstructive sleep apnea: putative pathways to the cardiovascular complications. *Antioxid Redox Signal* **10,** 755-768.

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR & Davisson RL. (2002). Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* **91,** 1038-1045.

Zimmerman MC, Lazartigues E, Sharma RV & Davisson RL. (2004). Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res* **95,** 210-216.

Zubcevic J, Waki H, Raizada MK & Paton JF. (2011). Autonomic-immune-vascular interaction: an emerging concept for neurogenic hypertension. *Hypertension* **57,** 1026-1033.

Zucker IH. (2006). Novel mechanisms of sympathetic regulation in chronic heart failure. *Hypertension* **48,** 1005-1011.

Figure Legends

Figure 1. (A) Summary data of ESR amplitude response to an Antioxidant Cocktail (AOX) during baseline and two workloads of dynamic exercise (EX120) and (EX150). (B) Representative ESR spectra from blood collected from one subject that received either Placebo or Antioxidant Cocktail (AOX CT) at rest and during exercise workload (EX120). Values are means ± SE; Placebo (PL); *****denotes $P \leq 0.05$ between treatment conditions; † denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition.

Figure 2. (A) MSNA burst frequency (bursts/minute, top left), (B) incidence (bursts/100 heart beats, bottom left) and (C) Mean arterial pressures (MAP) at rest and during both exercise workloads (EX120) and (EX150). Values are means ± SE; Placebo (PL) and Antioxidant Cocktail (AOX); *denotes $P \leq 0.05$ between treatment conditions; † denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition.

Figure 3. Muscle sympathetic activity (MSNA, arbitrary units (a.u.)) and arterial blood pressure (BP) tracings are shown for one representative subject during baseline (A) and both exercise workloads (B) EX120, (C) EX150 and under both conditions placebo (PL) and antioxidant cocktail (AOX).

Figure 4. Mean responses of blood pressure elected by perturbations of the carotid sinus baroreceptors at rest ((shaded symbols), ● Placebo (PL); ▼ Antioxidant Cocktail (AOX)) and during both dynamic exercise workloads (A) EX120 and (B) EX150 ((open symbols), ○ Placebo (PL); ∇ Antioxidant Cocktail (AOX)); Arrows indicate the directional effect of AOX on baroreflex resetting compared with placebo. Data were fitted with the logistic function to represent the mean response across each condition. Values are means ± SE

Table 1. Hemodynamic responses to antioxidants and exercise

	Baseline		Exercise 120 (EX120)		Exercise 150 (EX150)	
Variable	PL	AOX	PL	AOX	PL	AOX
HR, beats/min	64 ± 1.9	63 ± 2.3	117 ± 2.3†	111 ± 1.6*†	145 ± 2.4†‡	139 ± 2.6*†‡
SV, ml	83 ± 5.1	77 ± 4.5	124 ± 5.7†	118 ± 6.1†	118 ± 6.1†	110 ± 5.6†
Q, l/min	5.2 ± 0.3	4.8 ± 0.3	14.4 ± 0.6†	13.1 ± 0.8†	16.5 ± 0.6†‡	15.2 ± 0.8†‡
SAP, mmHg	136 ± 4	125 ± 2*	171 ± 3†	162 ± 4*†	162 ± 4†‡	154 ± 5*†
DAP, mmHg	76 ± 2	73 ± 1	86 ± 3†	85 ± 2†	84 ± 2†	81 ± 2†
MAP, mmHg	98 ± 2	92 ± 1*	116 ± 3†	111 ± 3*†	114 ± 3†	109 ± 2*†

Values are means ± SE; n=12; Placebo (PL) and Antioxidant Cocktail (AOX); HR (heart rate); SV (stroke volume); Q (cardiac output); SAP, DAP, MAP (systolic, diastolic, and mean arterial pressures). * denotes $P \leq 0.05$ between treatment conditions; † denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition

Table 2. Carotid-MAP baroreflex function curve parameter responses to antioxidants and exercise

	Baseline		Exercise 120 (EX120)		Exercise 150 (EX150)	
Variable	PL	AOX	PL	AOX	PL	AOX
Response Range, mmHg	21 ± 3	14 ± 2*	25 ± 3	19 ± 1 *	23 ± 3	17 ± 1*
Max Gain, mmHg/mmHg	-0.6 ± 0.1	-0.6 ± 0.1	-0.9 ± 0.1	-0.8 ± 0.1	-0.8 ± 0.1	-0.8 ± 0.1
Operating Point, mmHg	99 ± 3	94 ± 2*	114 ± 3†	108 ± 3*†	109 ± 3†	104 ± 3*†
Threshold, mmHg	65 ± 7	69 ± 2	88 ± 6†	87 ± 2†	89 ± 5†	87 ± 3†
Saturation, mmHg	123 ± 5	106 ± 2*	137 ± 5†	126 ± 4*†	131 ± 4†	122 ± 4*†
Min. MAP Response, mmHg	90 ± 3	90 ± 3	101 ± 3†	100 ± 3 †	97 ± 4†	97 ± 2†

Values are means ± SE; n=8; Placebo (PL) and Antioxidant Cocktail (AOX); response range equals maximum minus minimum mean arterial pressure (MAP) response. * denotes $P \leq 0.05$ between treatment conditions; † denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition

Figure 1

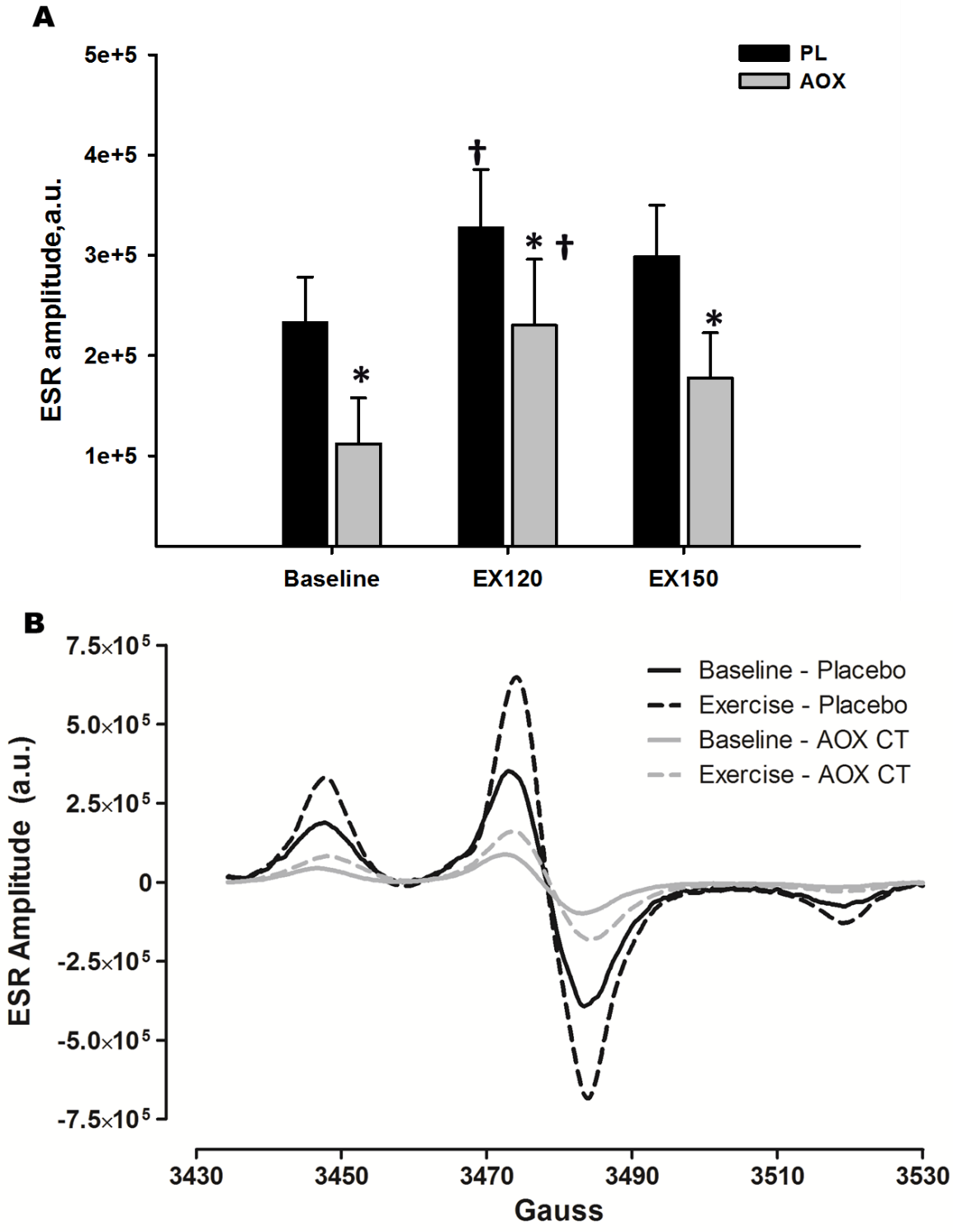

A
PL
AOX
ESR amplitude,a.u.
5e+5
4e+5
3e+5
2e+5
1e+5
Baseline
EX120
EX150
B
Baseline - Placebo
Exercise - Placebo
Baseline - AOX CT
Exercise - AOX CT
ESR Amplitude (a.u.)
7.5×10⁵
5.0×10⁵
2.5×10⁵
0
-2.5×10⁵
-5.0×10⁵
-7.5×10⁵
3430
3450
3470
3490
3510
3530
Gauss

Figure 2

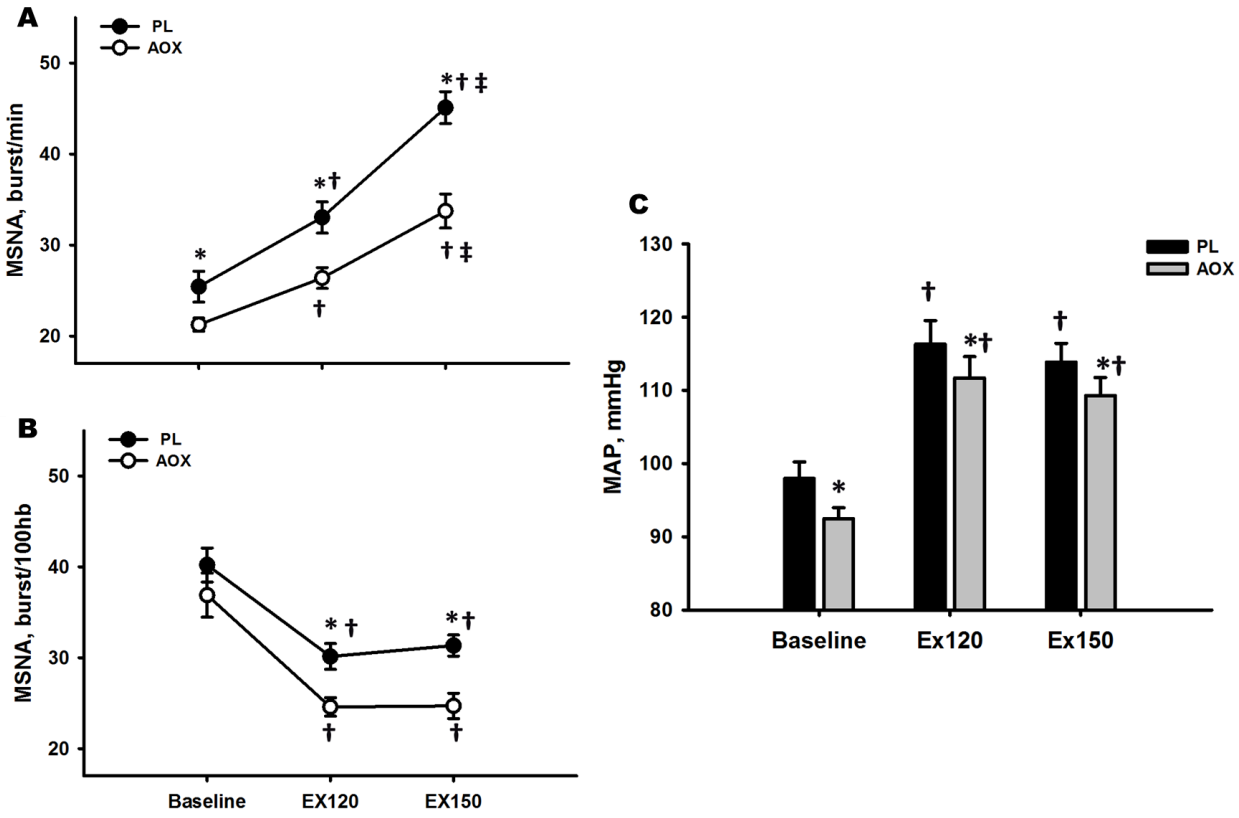

A
PL
AOX
MSNA, burst/min
50
40
30
20
*
*†
*† ‡
† ‡
†
B
PL
AOX
MSNA, burst/100hb
50
40
30
20
* †
*†
†
†
Baseline
EX120
EX150
C
PL
AOX
MAP, mmHg
130
120
110
100
90
80
*
†
*†
†
*†
Baseline
Ex120
Ex150

Figure 3

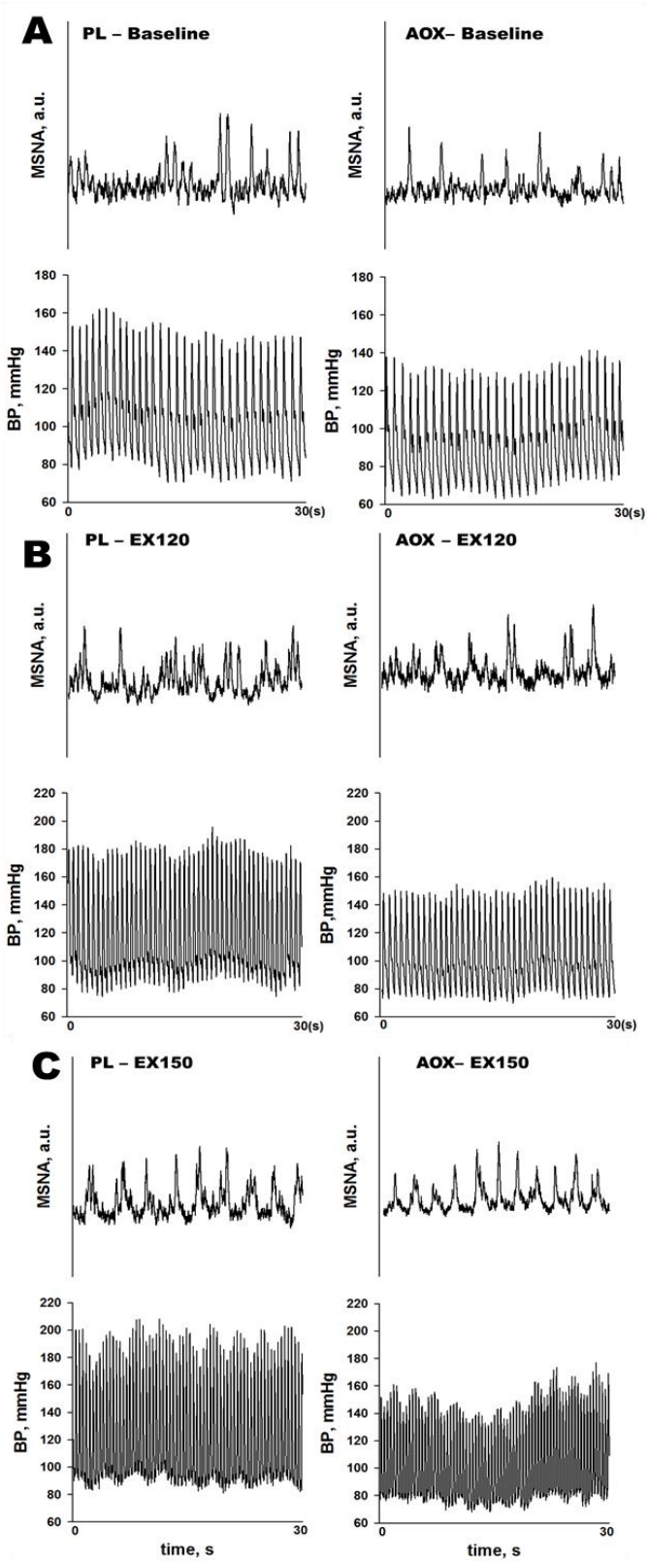
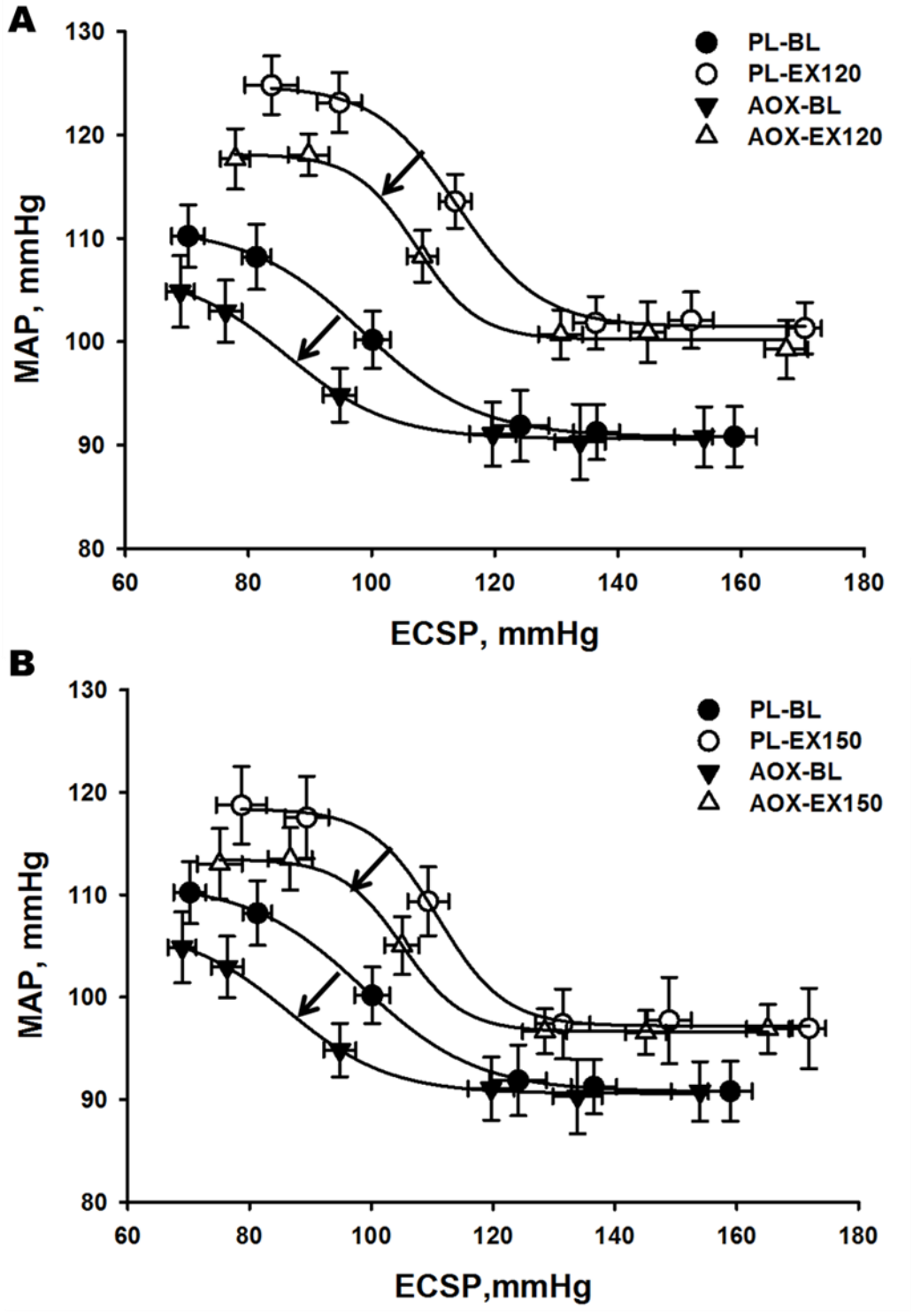

A
PL – Baseline
AOX– Baseline
MSNA, a.u.
BP, mmHg
B
PL – EX120
AOX – EX120
C
PL – EX150
AOX– EX150
0
30(s)
30
time, s

Figure 4


A
MAP, mmHg
ECSP, mmHg
PL-BL
PL-EX120
AOX-BL
AOX-EX120
B
MAP, mmHg
ECSP,mmHg
PL-BL
PL-EX150
AOX-BL
AOX-EX150

CHAPTER III

The role of angiotensin II in the exercise induced resetting of the arterial baroreflex during dynamic exercise

Gilbert Moralez[1], Noah P. Jouett[1], Jun Tian[2], Matthew C.Zimmerman[2] and Peter B. Raven[1]

[1] *Institute for Cardiovascular and Metabolic Disease, University of North Texas Health Science Center, Fort Worth, TX*

[2]*Department of Cellular and Integrative Physiology, University of Nebraska Medical Ctr., Omaha, NE*

ABSTRACT

During exercise, there is a physiological increase in angiotensin II (Ang II) and muscle sympathetic nerve activity (MSNA) in direct relation to increasing exercise intensity. We tested the hypothesis that the exercise induced increase in Ang II linked FR production, MSNA activity and arterial baroreflex ABR) resetting during exercise is located within the brain. Healthy subjects performed three randomly ordered trials of 70° upright back-supported dynamic leg cycling after ingestion of placebo (PL) and two different lipid soluble angiotensin converting enzyme inhibitors (ACEi), Perindopril (PER) - highly lipid soluble; Captopril (CAP) non-lipid soluble)) and/or placebo (PL). Repeated measurements of whole venous blood (N=8), MSNA (N=7), and arterial blood pressures (N=14) were obtained at rest and during steady-state heavy intensity exercise at heart rates (HR) of 120 beats/minute (E120) and HR of 150 beats/minute (E150). ABR function curves were modeled at rest and during cycling exercise. Peripheral venous superoxide concentrations as measured by electron spin resonance (ESR) spectroscopy were not significantly altered at rest ($P \geq 0.4$) and during exercise by the ACE inhibitors ($P \geq 0.3$). Likewise, baseline MSNA and MAP were unchanged at rest ($P \geq 0.1$; $P \geq 0.8$ respectively). However, during both exercise workloads central acting PER attenuated the increases in MSNA and MAP compared to PL ($P < 0.05$). Arterial pressures at the Operating Point (OP) and threshold (THR) pressures were decreased with PER from baseline to EX120 (i.e. resetting) with no further changes in the OP pressure during EX150 under all three conditions. From these data we conclude that centrally acting PER attenuated the central increase in the exercise induced Ang II linked free radical production enabling an increased central NO activity induced reduction in MSNA during heavy and very heavy intensity exercise.

INTRODUCTION

Short-term adjustments of arterial blood pressure (ABP) in response to abrupt changes in blood volume, cardiac output and peripheral resistance such as those that occur during exercise are affectively buffered by the activation of the arterial baroreflex (ABR). During dynamic exercise, a physiologic hypertension coexists with tachycardia which is essential to maintain increased cardiac output and to provide appropriate delivery of oxygen to working muscles (Mitchell, 1990; Rowell & O'Leary, 1990). It has been established by a number of investigators that activation of central command (CC) and/or the exercise pressor reflex (EPR) is essential for the progressive physiological resetting of the reference "Operating Point (OP)" pressures of the arterial baroreflex (ABR) in direct relation to the progressive increase in exercise intensity (Potts *et al.*, 1993; Norton *et al.*, 1999; Fadel & Raven, 2012) . The resetting of the ABR enables the observed parallel increases in heart rate (HR) and arterial blood pressure (ABP) with progressive increases in exercise intensity. Furthermore, during dynamic exercise, the physiologic production of angiotensin II (Ang II) and its related generation of free radicals (FR) are increased both centrally and peripherally and are linearly related to exercise intensity (Fallo, 1993; Bailey *et al.*, 2004; Shim *et al.*, 2008; Bailey *et al.*, 2011; Powers *et al.*, 2016). Exacerbated increases in central oxidative stress are implicated in impaired autonomic regulation of cardiovascular function associated with aging (Monahan *et al.*, 2004), hypertension (Grassi *et al.*, 2004), chronic heart failure (Nightingale *et al.*, 2003), and obstructive sleep apnea (Yamauchi & Kimura, 2008). Furthermore, a body of evidence suggests that essential hypertension has a central neurogenic origin related to FRs via Ang II linked FR production (Grobe *et al.*, 2008; Datla & Griendling, 2010). Physiologically central sympathetic outflow is inhibited by central nitric oxide (NO) (Aslan *et al.*, 2001) within the NTS and RVLM (Jiang *et al.*,1996). Recently it

has been established that central NO is scavenged by centrally generated FRs (Paton & Waki, 2009; Waki *et al.*, 2011), thereby, allowing greater central sympathetic outflow (Waki *et al.*, 2008; Fisher & Fadel, 2010). Furthermore, we have identified that both the combined central and peripheral FRs play a pivotal role in the exercise related resetting of the arterial baroreflex control of ABP and muscle sympathetic nerve activity (MSNA) in healthy subjects. We demonstrated that a global anti-oxidant cocktail (CoQ10, Vitamin E and Vitamin C) reduced ABR-OP pressure and MSNA during exercise to a greater extent compared to a placebo control. However, since we utilized a global anti-oxidant cocktail (acting both centrally and peripherally) we were unable to: i) unequivocally identify the major site of baroreflex resetting as being a single FR related mechanism within the central nervous system (CNS); and/or ii.) determine the role of Ang II linked FR production in the exercise-induced ABR resetting. Therefore, to address these questions we investigated the hypothesis that the Ang II linked exercise intensity dependent production of FRs within the brain was related to ABR-OP pressure resetting during exercise. We compared ABR-OP pressure resetting from rest to exercise without (placebo-PL) and with pharmacological inhibition of the angiotensin converting enzyme (ACEi) using effective equivalent doses (Agabiti-Rosei *et al.*, 1992) of: i) a global 4mg dose of Perindopril (PER), a highly lipid soluble ACEi that crosses the blood brain barrier (BBB); and ii) a peripheral 25 mg dose of Captopril (CAP), a non-lipid soluble ACEi that does not cross the BBB.

METHODS

***Subjects*:** 11 males (24 ±2 yrs,181±7 cm, 82± 5 kg) and 3 females (24 ±1 yrs,163 ± 5 cm, 67 ± 3 kg) volunteered for this study. Subjects were deemed healthy via General Health Questionnaire, were not taking any medications and did not smoke. Subjects were asked to

abstain from caffeine, exercise and alcohol 24 h before each experimental session due to possible influences on cardiovascular regulatory mechanisms. Subjects also received familiarization training with the experimental protocol and procedures before any experiments were performed. Written informed consents was obtained from all subjects. Female subjects were screened with an over-the-counter pregnancy test to ensure that they were not pregnant on the day of testing. Due to the potential effect of estrogen and progesterone on autonomic neural regulation of the cardiorespiratory systems, all female subjects not on hormonal contraception were tested in the early follicular (low hormone) phase of their menstrual cycle; female subjects on hormonal contraception were tested during the low hormone or placebo phase of their contraception cycle (i.e., days 1-4 post menses). Our study conformed to guidelines set forth by the Declaration of Helsinki, and was approved (IRB # 2014-062) by the Institutional Review Board for the Protection of Human Subjects in Research at the University of North Texas Health Science Center.

Experimental protocol: Subjects visited the laboratory on four separate occasions once for an orientation day and the other three visits were randomized among the three experimental pharmacologic protocols (PL, PER and CAP). Experimental protocols were separated by at least one week, during which time subjects were instructed to maintain their normal exercise and diet regimen. Each subject performed an orientation protocol to familiarize themselves with the laboratory and its equipment to reduce any potential 'white-coat' effects on measured variables. The subjects then underwent a graded exercise test (GXT) on a 70̊ back supported semi-recumbent cycle ergometer to determine their exercise tolerance and workload for steady state exercise at HRs of 120 and 150 beats/min. These exercise work intensities are considered to be heavy and very heavy work, respectively, (Åstrand & Rodahl, 1986). Ultrasound and blood flow

Doppler measurements were used to visualize the anatomy and flow of the carotid arteries within the neck to ensure that the bilateral vascularization of the neck is intact and free of atherosclerotic plaques. If no abnormalities were noted blood vessel anatomy or blood flow, the subject practiced wearing the neck collar and underwent 2-3 trials of NP/NS at rest and during mild exercise. On completion of the Orientation protocol, the subjects were assigned to one of three experimental groups for their first experiment: i) PL; ii) PER; iii) CAP in a counterbalance design. Furthermore, because in rare instances passive anaphylaxis has been observed following ACEi, hence, our counterbalanced order always present CAP ahead of PER because the time for CAP to achieve peak blood concentrations occurs in the1hr the subject is being instrumented in the laboratory.

ACE inhibitors (ACEi): Comparison dosages for the ACE inhibitors were determined by our board certified cardiologist consultant and were set as the acute dose required for approximately equal changes in blood pressure in clinical applications based on previous studies (Lees & Reid, 1987; Agabiti-Rosei *et al.*, 1992; Jankowski *et al.*, 1995; Chik *et al.*, 2010). Furthermore, single dose studies in healthy human subject have identified that plasma angiotensin II concentrations are reduced by 31% with both 25mg CAP measured at 1 hour post-ingestion (Hollenberg *et al.*, 1981) and 4mg of PER measured at hour 4 post-ingestion (Bussien *et al.*, 1986).

Instrumentation and exercise protocol. Once subjects successfully completed orientation day they were invited back to participate in the experimental protocol. Each experimental protocol was performed at the same time of day for each subject to account for the influence of circadian rhythms on the measured variables. After ingestion of either the PL or ACEi capsules, subjects were instrumented with a 3-lead ECG (Hewlett-Packard, Inc.) to monitor heart rate, beat-to-beat arterial blood pressure measurements were obtained from the finger by photoplethysmography

(Finometer, Finapres Medical Systems, Amsterdam, The Netherlands), and multiunit muscle sympathetic nerve activity (MSNA) was recorded directly from the radial nerve at the spiral groove in the upper arm using standardized (Hagbarth & Vallbo, 1968; Wallin et al., 1994; Vallbo et al., 2004) and ultrasound-guided microneurography techniques (Curry & Charkoudian, 2011). Additionally, an intravenous catheter was inserted into an antecubital fossa vein to withdraw 5 mL samples of blood for the measurement of peripheral superoxide concentrations.

Subjects were positioned on the same stationary bicycle they performed the GXT, in a 70^0 back supported semi-recumbent position with the legs extended parallel with the floor. The experimental protocol on each Experimental Day consisted of three stages:1) Baseline (resting), 2) Stage E120 (exercise at HR of 120 beats), 3) Stage E150 (exercise at HR of 150 beats/min). Data were collected for 10 minutes at baseline and 10 min for stages for E120 and E150 once the subject achieved steady state measures of HR and ABP. Upon reaching steady state the NP/NS protocol was performed to identify changes in HR and ABP that enabled our modeling of ABR function curves, as previously described (Potts *et al.*, 1993; Norton *et al.*, 1999), Furthermore, venous blood samples were obtained after each steady state stage of rest, E120 and E150 before the NP/NS protocol. Subjects rested in the semi-recumbent position for at least 10-min to establish pre-exercise baseline values and a blood sample was obtained at the end of baseline. After collecting the resting data, the subject began to exercise with a zero load and a slow pedal frequency pedaling exercise was performed on a computer-controlled magnetically braked ergometer (Intellifit, Houston, Texas). Both the pedal frequency and the intensity of exercise were progressively increased to a maximum of 60 rpm over the 5 min to achieve a constant workload at a HR of 120 beats/min. After attaining steady-state circulatory and respiratory responses (~ 5 minutes during each exercise workload) hemodynamic data were collected for 5

minutes. The subject continued to perform exercise at this constant workload for approximately 15 min in order to perform the NP/NS protocol for determining the ABR function curves established by (Potts *et al.*, 1993) and (Norton *et al.*, 1999). After completion of Stage E120 the subject continued to exercise as the workload was slowly increased to the workload determined on their Orientation Day to achieve and maintain a HR of 150 beats/min which also lasted 20 min. The two exercise workloads EX120 (heavy) and EX150 (very heavy (Åstrand & Rodahl, 1986)) were performed continuously approximately 20 minutes each for a total of 40 minutes.

ABR stimulus-response function and curves: Assessment of carotid baroreflex function was performed using our customized computer controlled variable pressure neck collar and protocol that have been previously established (Pawelczyk et al.,1989; Potts et al. 1993(Norton *et al.*, 1999; Fadel, 2008; Raven, 2012). Arterial baroreflex stimulus-response curves for MAP were modeled for each subject to a four-parameter logistic function described by Kent et al. (Kent *et al.*, 1972) and is as follows:

$$MAP = A_1 \{1 + \exp [A_2 (ECSP - A_3)] \}^{-1} + A_4$$

MAP is the dependent variable, ECSP is the Estimated Carotid Sinus Pressure, A_1 is the range (i.e. maximum – minimum), A_2 is the gain coefficient (average of the slope of the function), A_3 is the value at the centering point (i.e. point of maximum gain) and A_4 is the minimum response of MAP. Furthermore, the carotid sinus threshold pressures (THR-the point where no further increases in MAP were elicited by reductions in ECSP) and saturation (SAT) pressures (the point where no further decrease in MAP were elicited by increases in ECSP and

were calculated using the following equations, (the definitions of the variables A_1-A_4 are provided above) (Potts *et al.*, 1993; McDowall & Dampney, 2006):

Saturation: $A_3+2.944/A_2$

Threshold: $A_3-2.944/A_2$

The operating point (OP) of each curve was defined as a stimulus ESCP and was averaged over the respective time point (e.g. rest, EX120, EX150) after steady-state circulatory responses were obtained. Hence, for each subject and condition, the OP, SAT and THR pressures were calculated and compared between and across subjects.

Measurement of Free Radicals: Electron spin resonance (ESR) spectroscopy was utilized to measure peripheral free radicals activity (in particular superoxide ($[O_2\cdot^-]$) directly in venous blood samples. Triplicate 200 μL whole blood samples for each time point (baseline, EX120, and EX150) were incubated for 15 min in a buffer solution: 3.5 mM deferoxamine methanesulfonate salt (DF), 9.08 mM of diethyldithiocarbamic acid sodium (DETC) and Krebs-HEPES buffer (Noxygen Science Transfer & Diagnostics GmbH, Elzach, Germany) containing methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH) as spin probe, which preferentially detects $O_2\cdot^-$ (Deo *et al.*, 2012; Vianna *et al.*, 2015). 50 μl samples of whole blood for each time point in duplicate were then loaded into a 1-cc syringe and flash frozen using liquid nitrogen between buffer solutions to form a continuous frozen plug. Samples were then stored at −80°C and shipped to the University of Nebraska Medical Center's ESR Spectroscopy Core for analysis (Deo *et al.*, 2012; Vianna *et al.*, 2015). ESR amplitude was measured using a Bruker e-Scan ESR Spectrometer and was averaged for all triplicate data to generate an individual subject average for each time point.

Data acquisition and analysis: Electronically recorded data were sampled at 500Hz and recorded to a computer via data acquisition software (WINDAQ, Dataq Instruments, Akron, OH). The data were then analyzed using a commercially available biomedical analysis software program (WinCPRS, Absolute Aliens, Turku, Finland). Data from the last 2 minutes of the 10-minute baseline were analyzed to establish baseline values. Additionally, exercise data were analyzed in 2 minute epochs during the first workload (EX120) and second workload (EX150) after 3 minutes of steady-state exercise. R-waves generated from the ECG were detected and marked at their occurrence in time. Systolic and diastolic pressures (SAP, DAP) were marked from the arterial pressure waveforms and MAP was automatically calculated as the area under the arterial pressure waveforms, via the WinCPRS software. Beat-to-beat stroke volume (SV) were estimated using the pulse contour method (Jansen et al., 1990). Multiunit muscle sympathetic nerve activity (MSNA) signals were band-pass filtered (100–2000 Hz), and integrated (time constant, 0.1 s) to obtain mean voltage neurograms. Neurograms were subsequently imported into the aforementioned biomedical analysis software program which has the capability of detecting bursts of MSNA. The software detects burst of MSNA based on two primary criteria (1) pulse synchronous spontaneous bursts with signal-to-noise ratios of approximately 3 :1; and (2) reflex latencies from preceding R-waves of approximately 0.9 s (Wallin et al., 1994; Cooke et al., 2009).Furthermore, the same experienced microneurographer (GM) manually checked the computerized burst detection results. MSNA was expressed as bursts per minute (burst frequency, BF) and as bursts per 100 heart beats (burst incidence, BI). Due to the baseline fluctuations of the neurograms that occur during heavy intensity exercise and inherent to our repeated measures design, the amplitude and area of sympathetic bursts were not expressed. As the amplitude and area of sympathetic bursts varies within and between subjects

due to electrode position we cannot verify that position of the electrode within a subject are the same on two different occasions. Therefore, we concluded that it would be, inappropriate to express the amplitude and area of sympathetic bursts for this particular protocol (White *et al.*, 2015).

Statistical analysis: All data were analyzed with (SigmaPlot, Systat Software Inc., California, USA). For assessment of differences among the mean values of interest between baseline and exercise, a three (treatment; PL, CAP, and PER) by three (time; baseline, EX120 and EX150) factorial ANOVA for repeated measures were implemented. A Student-Newman-Keuls post hoc test was conducted to determine differences between groups. Data are presented as means ± SE and statistical significance was determined as $p < 0.05$

RESULTS

Arterial BP responses to ACEi during rest and exercise: Oral ingestion of the ACEi did not reduce plasma superoxide concentrations [O2·−] at rest or during the exercise protocol (N=8, $P \geq 0.3$) (Fig 1A). The [O2·−], however, did increase from baseline to E120 in all three treatment groups ($P \leq 0.05$) and remained elevated throughout E150 with no further increases in [O2·−] with the increased workload ($P \geq 0.16$, Fig 1A) for PL and CAP. On the other hand, PER [O2·−] continued to increase from E120 to E150 ($P = 0.06$ Fig 1A). Furthermore, the percent increase in [O2·−] illustrates a significant rise for PL and CAP from rest to E120 ($P < 0.05$) but not for PER ($P = 0.11$; figure 1B).

No changes in ABP were observed at rest with the oral administration of either ACEi (N=14, $P \geq 0.5$). However, systolic arterial pressures (SAP) and diastolic arterial pressures (DAP) were reduced with PER during E120 when compared to PL ($P < 0.05$). Steady-state exercise

MAPs (the OP pressure) measured during the PER condition were significantly lower during E120 when compared to both PL and CAP ($P \leq 0.05$, Fig. 2). When expressed as a percent change from baseline PER attenuated DAP and MAP compared to PL and CAP ($P \leq 0.05$, Fig. 2 B & C). The increase in workload to E150 did not result in a significant rise in ABP under all three conditions ($P \geq 0.1$). However, similar reductions in DAP and MAP were observed with PER during E150 when compared to PL ($P \leq 0.05$, Fig. 2 B & C). In addition, leg cycling exercise elicited significant increases in HR, SV and Q under both conditions compared to baseline and progressively increased from EX120 to EX150 with the exception of SV with ACEi (CAP, P =0.9); PER, P =0.9). A summary of the hemodynamic variables is shown in table 1.

MSNA responses to dynamic exercise with and without ACEi:

Fig. 3 is a comparison summary of a representative subject's MSNA and ABP recordings comparing baseline, EX120 and EX150 under all three conditions. ACEi did not result in a notable change in MSNA burst frequency (BF) at rest (N=7; $P \geq 0.2$ (figure 4A)). However, the repeated measures ANOVA identified an interaction of MSNA BF with PL, CAP and PER at different exercise conditions of EX120 and EX150. Compared to placebo and captopril, the perindopril BF of MSNA during E120 was decreased by 6 bursts/min and 11 bursts/min at EX150 ($P \leq 0.03$; N=7 (Fig 4A)). Dynamic leg cycling exercise provoked significant increases in MSNA (BF) from rest to EX120 under all conditions (figure 4A) ($P < 0.01$)). In addition, MSNA-BF at EX150 was significantly increased by with PL and CAP above the BF in the EX120 in PL but in PER conditions ($P < 0.01$). The attenuation of MSNA (BF) is illustrated as percent change from baseline in figure 4B.

Arterial baroreflex control of mean arterial pressure with and without ACEi: The stimulus-response relationship for the arterial baroreflex control of MAP at rest and during both workloads of steady-state dynamic leg cycling exercise is presented in figures 5A, 5B and 5C. For a clearer interpretation of the data each stimulus response curve was plotted individually on one graph for each condition at baseline (Fig 5A), workload E120 (Fig 5B) and workload E150 (Fig 5C). Furthermore, logistic parameters describing the ABR-MAP relationship are shown in table 2. Oral ingestion of ACEi did not change the ABR-MAP parameters at baseline ($P \geq 0.10$).However, throughout dynamic exercise ACEi did alter the ABR-MAP response range during E120 ($P \geq 0.05$) but not E150 when compared to baseline. In addition, the Gmax at the centering point (CP pressure = where an equal pressor and depressor response occurs) of the ABR-MAP stimulus response relationship was similar at rest and during both exercise workloads and was not affected by ACEi throughout the protocol ($P \geq 0.50$; table 2). On the other hand, arterial pressures at the OP and THR pressures were decreased with PER from baseline to EX120 (i.e. resetting) with no further changes in the OP pressure during EX150 under all three conditions. Moreover, PER significantly decreased the OP pressures at EX120 and EX150 indicating that central Ang II may be involved in establishing the OP pressure of the ABR during dynamic exercise (figures 5B and 5C).

DISCUSSION

The data obtained in this investigation provides new insights into the role of central Ang II and its influence on the arterial baroreflex resetting of MAP and MSNA during dynamic leg cycling exercise in humans. The contribution of the key inputs to ABR resetting have been established (Smith *et al.*, 1976; Gallagher *et al.*, 2001a, b; Ogoh *et al.*, 2007) .,i.e. increases in input to the NTS from CC and EPR will accentuate sympathetic outflow and increase blood

pressure (Victor *et al.*, 1987; Potts, 2006), whereas increases in baroreceptor afferent input to the NTS will restrain sympathetic outflow and decrease blood pressure (Sheriff *et al.*, 1990). However, this investigation identified a central Ang II production signaling mechanism linked to dynamic exercise intensity that increases central sympathetic neural (SNA) outflow with increases in exercise intensity.

Recent animal data has implicated NO as a modulator of central SNA outflow. For example, introduction of NO centrally dampens sympathetic outflow (Patel *et al.*, 2001; Gao *et al.*, 2011), and there is a growing body of evidence that indicates that central NO is scavenged by centrally generated ROS (Paton & Waki, 2009; Waki *et al.*, 2011; Leal *et al.*, 2012; Leal *et al.*, 2013). Hence, during dynamic exercise, increases in centrally generated FRs formed by increased intensity-related oxidative metabolism and central Ang II linked FR production (Fallo, 1993; Bailey *et al.*, 2004; Shim *et al.*, 2008; Bailey *et al.*, 2011) suggest that FRs are likely candidate signaling molecules involved in allowing the ABR to progressively reset with increasing exercise intensity. In this investigation we demonstrated that, indeed, central Ang II production is a prerequisite for the ABR-MAP resetting and hence, facilitates the increases in central SNA outflow directly related to increases in exercise intensity. By utilizing the permeability kinetics of PER, we demonstrated that its central inhibition of Ang II production resulted in the greatest attenuation of the exercise-induced resetting of ABR control of MSNA and MAP, when compared to PL and CAP (Fig 4 & 5). Furthermore, even though MAP decreased in the CAP condition compared to PL that can be attributed to systemic ACE inhibition, there was no decrease in MSNA during exercise (Fig 2 & 5). In fact, MSNA was slightly elevated with CAP compared to placebo suggesting that the systemic effect of CAP elicited hypotension that is sensed by the arterial baroreceptor mediated reflex response in

MSNA. However, this reflex response was not observed during the PER condition indicating that it was the central blocking effect of PER that resulted in a manifest reduction of the ABR-OP pressure (Fig 5).

Human studies utilizing selective anesthetic (Fentanyl) blockade of respiratory muscle afferents (Secher & Amann, 2012) and epidural anesthesia (Lidocaine) of leg skeletal muscle afferents (Smith *et al.*, 2003) during exercise have yielded similar results as those reported in the current investigation. For example, epidural blockade causes the carotid baroreflex control of MAP function curves to reset downward to a lower OP pressure (Smith *et al.*, 2003) similar to the effect PER produced in the present investigation (Fig 5). In the present study, analyses of the CBR-MAP reflex function curves during exercise, particular E120 (Fig 5B, table 2) identified an attenuation of the MAP-OP and threshold pressures from rest to exercise when compared to PL and CAP. In figures 4A & 5B, the central and peripheral acting ACEi, PER, selectively reduces the OP pressure of MAP and MSNA during exercise compared to both placebo and the peripherally acting ACEi, CAP. We suggest that the differences in the MAP-OP and threshold pressures of the CBR-MAP reflex function curves indicate : i) the systemic effect of PER; and ii) the suppression of central sympathetic outflow during exercise due to PER (Fig 4 and Fig 5).

Perspectives: In humans, we proposed to identify the role of central and peripheral Ang II linked FR production on exercise induced ABR resetting by using the differential lipid solubility of effective dose equivalents of ACE inhibitors. To our knowledge, the use of different lipid soluble ACEs on ABR resetting during exercise has not been demonstrated previously. The findings of the present investigation confirmed that central Ang II has a physiologic role in ABR-OP pressure resetting and steady-state MSNA during exercise.

Clinically, impaired autonomic control of SNA leads to poor prognosis in cardiovascular morbidities such as hypertension, left ventricular hypertrophy, chronic heart failure, obstructive sleep apnea, diabetes, obesity and normal aging (Narkiewicz *et al.*, 1998; Zucker *et al.*, 2001; Nightingale *et al.*, 2003; Monahan *et al.*, 2004; Osborn *et al.*, 2005; Xue *et al.*, 2005; Paton *et al.*, 2008). The results of the present investigation may provide physicians a clear choice of treatment options dependent upon the primary central or peripheral cause of exercise induced hypertension (EIHt). Hence, it is logical to investigate inhibition of central Ang II blockade as an adjunctive therapy for the exercise induced augmented increase in blood pressure of EIHt patients.

Limitations:

In this current investigation, circulating Ang II was not measured during exercise, before or after ACEi ingestion. It is well known, however, that circulating Ang II inhibits baroreflex activity through interactions with AT_1 receptors in the cicumventricular organs (CVOs) (Sanderford & Bishop, 2002; Tan *et al.*, 2007). The subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) are CVOs which lack a functional BBB and are sensitive to circulating Ang II (Osborn *et al.*, 2007). The SFO and OVLT innervate the median preoptic nucleus (MnPO) (Johnson & Gross, 1993) which projects to the PVN (Stocker & Toney, 2005) and modulates sympathetic outflow through the NTS and RVLM. However in this current investigation, the doses of ACEi that we utilized have been reported, , to reduce plasma Ang II concentration by 31% with both 25mg CAP measured at 1 hour post-ingestion (Hollenberg *et al.*, 1981) and 4mg of PER measured at hour 4 post-ingestion (Bussien *et al.*, 1986).Therefore, by administering doses that will attenuate the rise in circulating Ang II to the same extent during exercise any change in central SNA outflow can be contributed to the lipid soluble agent acting centrally on endogenous Ang II.

The overactive renin–angiotensin system (RAS) may be controlled by decreasing Ang II formation with renin or ACE inhibitors (as in the current investigation), or by blocking the AT1 receptors with Angiotensin Receptor Blockers (ARBs) (Villapol & Saavedra, 2015). However, additional pathways have been proposed to interact with the classical RAS system. In particular production of Ang (1–7) directly from Ang I by neutral endopeptidase (NEP) and in turn, Ang (1–7) activates the Mas receptors and act as a counteregulatory axis to ACE/ANG II/ AT1R (Chappell, 2007; Ferreira *et al.*, 2010; Shah *et al.*, 2012). The ANG -(1-7) pathway is considered as an important core factor in cardiovascular pathophysiology (Chappell, 2007; Ferreira *et al.*, 2010). Studies have identified that activation of ANG-(1–7) includes vasorelaxation via the Mas receptor (Ren *et al.*, 2002; Santos *et al.*, 2003). Ren *et al.* (2002) suggested that Ang-(1-7)–induced vasodilatation may be caused by production of NO. In the current investigation the activation of the Ang-(1-7) is unknown; however, the activation of this pathway may lead to an increase in NO therefore exacerbating the central inhibitory actions of NO.

A wide body of literature has identified sex differences in neural vascular control in men and women. Recently, Hart & Charkoudian identify that in young women the influence of the β-adrenergic receptors significantly offset the relationship between MSNA and vasoconstriction (Hart & Charkoudian, 2014). The current study utilized both male and female volunteers, but the ratio of male to female subjects was large (11:3), so we did not anticipate significant differences in the overall physiological conclusions of the present study. Alternatively, in a study that would utilize equal numbers of male and female subjects, we would anticipate a blunted responsiveness of young females to adrenergic stimuli which would reduce MAP changes.

CONCLUSIONS

The question addressed in this investigation was whether or not the central Ang II formed during exercise is fundamental to the exercise intensity related ABR resetting. The main finding identifies that indeed central Ang II production plays a pivotal role in the full expression of the exercise related rightward and upward resetting of the ABR-OP pressure and the reflex control of central SNA outflow. From these data, we conclude that the centrally acting ACEi perindopril attenuated the exercise related increase in central Ang II and its free radical concentration resulting in an increased attenuation of central MSNA outflow during dynamic exercise most likely through a NO mediated mechanism (Campese *et al.*, 2004; Waki *et al.*, 2011).

REFERENCES

Agabiti-Rosei E, Ambrosioni E, Finardi G, Folino P, Gambassi G, Malini P, Marchesi E, Muiesan ML, Semplicini A & Pessina AC. (1992). Perindopril versus captopril: efficacy and acceptability in an Italian multicenter trial. *Am J Med* **92,** 79S-83S.

Aslan M, Ryan TM, Adler B, Townes TM, Parks DA, Thompson JA, Tousson A, Gladwin MT, Patel RP, Tarpey MM, Batinic-Haberle I, White CR & Freeman BA. (2001). Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Natl Acad Sci U S A* **98,** 15215-15220.

Åstrand P-O & Rodahl K. (1986). *Textbook of work physiology : physiological bases of exercise*. McGraw-Hill, New York ; London.

Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, Ogoh S, Ainslie PN, Lucchesi C, Rockenbauer A, Culcasi M & Pietri S. (2011). Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. *Experimental physiology* **96,** 1196-1207.

Bailey DM, Young IS, McEneny J, Lawrenson L, Kim J, Barden J & Richardson RS. (2004). Regulation of free radical outflow from an isolated muscle bed in exercising humans. *Am J Physiol Heart Circ Physiol* **287,** H1689-1699.

Bussien JP, d'Amore TF, Perret L, Porchet M, Nussberger J, Waeber B & Brunner HR. (1986). Single and repeated dosing of the converting enzyme inhibitor perindopril to normal subjects. *Clin Pharmacol Ther* **39,** 554-558.

Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z & Chiu J. (2004). Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* **287,** H695-703.

Chappell MC. (2007). Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1-7)-MAS receptor axis: more than regulation of blood pressure? *Hypertension* **50,** 596-599.

Chik Z, Basu RC, Pendek R, Lee TC & Mohamed Z. (2010). A bioequivalence comparison of two formulations of rifampicin (300- vs 150-mg capsules): An open-label, randomized, two-treatment, two-way crossover study in healthy volunteers. *Clinical therapeutics* **32,** 1822-1831.

Cooke WH, Rickards CA, Ryan KL, Kuusela TA & Convertino VA. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol* **587,** 4987-4999.

Curry TB & Charkoudian N. (2011). The use of real-time ultrasound in microneurography. *Auton Neurosci* **162,** 89-93.

Datla SR & Griendling KK. (2010). Reactive oxygen species, NADPH oxidases, and hypertension. *Hypertension* **56,** 325-330.

Deo SH, Fisher JP, Vianna LC, Kim A, Chockalingam A, Zimmerman MC, Zucker IH & Fadel PJ. (2012). Statin therapy lowers muscle sympathetic nerve activity and oxidative stress in patients with heart failure. *American journal of physiology Heart and circulatory physiology* **303,** H377-385.

Fadel PJ. (2008). Arterial baroreflex control of the peripheral vasculature in humans: rest and exercise. *Med Sci Sports Exerc* **40,** 2055-2062.

Fadel PJ & Raven PB. (2012). Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. *Experimental physiology* **97,** 39-50.

Fallo F. (1993). Renin-angiotensin-aldosterone system and physical exercise. *J Sports Med Phys Fitness* **33,** 306-312.

Ferreira AJ, Santos RA, Bradford CN, Mecca AP, Sumners C, Katovich MJ & Raizada MK. (2010). Therapeutic implications of the vasoprotective axis of the renin-angiotensin system in cardiovascular diseases. *Hypertension* **55,** 207-213.

Fisher JP & Fadel PJ. (2010). Therapeutic strategies for targeting excessive central sympathetic activation in human hypertension. *Exp Physiol* **95,** 572-580.

Gallagher KM, Fadel PJ, Stromstad M, Ide K, Smith SA, Querry RG, Raven PB & Secher NH. (2001a). Effects of exercise pressor reflex activation on carotid baroreflex function during exercise in humans. *J Physiol* **533,** 871-880.

Gallagher KM, Fadel PJ, Stromstad M, Ide K, Smith SA, Querry RG, Raven PB & Secher NH. (2001b). Effects of partial neuromuscular blockade on carotid baroreflex function during exercise in humans. *J Physiol* **533,** 861-870.

Gao J, Zhang H, Le KD, Chao J & Gao L. (2011). Activation of central angiotensin type 2 receptors suppresses norepinephrine excretion and blood pressure in conscious rats. *Am J Hypertens* **24,** 724-730.

Grassi G, Seravalle G, Dell'Oro R, Facchini A, Ilardo V & Mancia G. (2004). Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. *Journal of hypertension* **22,** 1747-1753.

Grobe JL, Xu D & Sigmund CD. (2008). An intracellular renin-angiotensin system in neurons: fact, hypothesis, or fantasy. *Physiology (Bethesda)* **23,** 187-193.

Hagbarth KE & Vallbo AB. (1968). Discharge characteristics of human muscle afferents during muscle stretch and contraction. *Exp Neurol* **22,** 674-694.

Hart EC & Charkoudian N. (2014). Sympathetic neural regulation of blood pressure: influences of sex and aging. *Physiology (Bethesda)* **29,** 8-15.

Hollenberg NK, Meggs LG, Williams GH, Katz J, Garnic JD & Harrington DP. (1981). Sodium intake and renal responses to captopril in normal man and in essential hypertension. *Kidney Int* **20,** 240-245.

Jankowski A, Skorek A, Krzysko K, Zarzycki PK, Ochocka RJ & Lamparczyk H. (1995). Captopril: determination in blood and pharmacokinetics after single oral dose. *Journal of pharmaceutical and biomedical analysis* **13,** 655-660.

Jansen JR, Wesseling KH, Settels JJ & Schreuder JJ. (1990). Continuous cardiac output monitoring by pulse contour during cardiac surgery. *Eur Heart J* **11 Suppl I,** 26-32.

Jiang H, Rummage JA, Stewart CA, Herriott MJ, Kolosova I, Kolosov M & Leu RW. (1996). Evidence for endogenous C1q modulates TNF-alpha receptor synthesis and autocrine binding of TNF-alpha associated with lipid A activation of murine macrophages for nitric oxide production. *Cell Immunol* **170,** 34-40.

Johnson AK & Gross PM. (1993). Sensory circumventricular organs and brain homeostatic pathways. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **7,** 678-686.

Kent BB, Drane JW, Blumenstein B & Manning JW. (1972). A mathematical model to assess changes in the baroreceptor reflex. *Cardiology* **57,** 295-310.

Leal AK, Mitchell JH & Smith SA. (2013). Treatment of muscle mechanoreflex dysfunction in hypertension: effects of L-arginine dialysis in the nucleus tractus solitarii. *Exp Physiol*.

Leal AK, Murphy MN, Iwamoto GA, Mitchell JH & Smith SA. (2012). A role for nitric oxide within the nucleus tractus solitarii in the development of muscle mechanoreflex dysfunction in hypertension. *Experimental physiology* **97,** 1292-1304.

Lees KR & Reid JL. (1987). Haemodynamic and humoral effects of oral perindopril, an angiotensin converting enzyme inhibitor, in man. *British journal of clinical pharmacology* **23,** 159-164.

McDowall LM & Dampney RA. (2006). Calculation of threshold and saturation points of sigmoidal baroreflex function curves. *Am J Physiol Heart Circ Physiol* **291,** H2003-2007.

Mitchell JH. (1990). J.B. Wolffe memorial lecture. Neural control of the circulation during exercise. *Med Sci Sports Exerc* **22,** 141-154.

Monahan KD, Eskurza I & Seals DR. (2004). Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men. *Am J Physiol Heart Circ Physiol* **286,** H2113-2117.

Narkiewicz K, Pesek CA, Kato M, Phillips BG, Davison DE & Somers VK. (1998). Baroreflex control of sympathetic nerve activity and heart rate in obstructive sleep apnea. *Hypertension* **32,** 1039-1043.

Nightingale AK, Blackman DJ, Field R, Glover NJ, Pegge N, Mumford C, Schmitt M, Ellis GR, Morris-Thurgood JA & Frenneaux MP. (2003). Role of nitric oxide and oxidative stress in baroreceptor dysfunction in patients with chronic heart failure. *Clin Sci (Lond)* **104,** 529-535.

Norton KH, Boushel R, Strange S, Saltin B & Raven PB. (1999). Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *J Appl Physiol (1985)* **87,** 332-338.

Ogoh S, Fisher JP, Fadel PJ & Raven PB. (2007). Increases in central blood volume modulate carotid baroreflex resetting during dynamic exercise in humans. *J Physiol* **581,** 405-418.

Osborn JW, Fink GD, Sved AF, Toney GM & Raizada MK. (2007). Circulating angiotensin II and dietary salt: converging signals for neurogenic hypertension. *Current hypertension reports* **9,** 228-235.

Osborn JW, Jacob F & Guzman P. (2005). A neural set point for the long-term control of arterial pressure: beyond the arterial baroreceptor reflex. *Am J Physiol Regul Integr Comp Physiol* **288,** R846-855.

Patel KP, Li YF & Hirooka Y. (2001). Role of nitric oxide in central sympathetic outflow. *Exp Biol Med (Maywood)* **226,** 814-824.

Paton JF & Waki H. (2009). Is neurogenic hypertension related to vascular inflammation of the brainstem? *Neurosci Biobehav Rev* **33,** 89-94.

Paton JF, Wang S, Polson JW & Kasparov S. (2008). Signalling across the blood brain barrier by angiotensin II: novel implications for neurogenic hypertension. *J Mol Med* **86,** 705-710.

Potts JT. (2006). Inhibitory neurotransmission in the nucleus tractus solitarii: implications for baroreflex resetting during exercise. *Exp Physiol* **91,** 59-72.

Potts JT, Shi XR & Raven PB. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol* **265,** H1928-1938.

Powers SK, Radak Z & Ji LL. (2016). Exercise-induced oxidative stress: past, present and future. *J Physiol*.

Raven PB. (2012). Neural control of the circulation: exercise. *Exp Physiol* **97,** 10-13.

Ren Y, Garvin JL & Carretero OA. (2002). Vasodilator action of angiotensin-(1-7) on isolated rabbit afferent arterioles. *Hypertension* **39,** 799-802.

Rowell LB & O'Leary DS. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J Appl Physiol (1985)* **69,** 407-418.

Sanderford MG & Bishop VS. (2002). Central mechanisms of acute ANG II modulation of arterial baroreflex control of renal sympathetic nerve activity. *American journal of physiology Heart and circulatory physiology* **282,** H1592-1602.

Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss HP, Speth R & Walther T. (2003). Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A* **100,** 8258-8263.

Secher NH & Amann M. (2012). Human investigations into the exercise pressor reflex. *Exp Physiol* **97,** 59-69.

Shah A, Oh YB, Lee SH, Lim JM & Kim SH. (2012). Angiotensin-(1-7) attenuates hypertension in exercise-trained renal hypertensive rats. *Am J Physiol Heart Circ Physiol* **302,** H2372-2380.

Sheriff DD, O'Leary DS, Scher AM & Rowell LB. (1990). Baroreflex attenuates pressor response to graded muscle ischemia in exercising dogs. *Am J Physiol* **258,** H305-310.

Shim CY, Ha JW, Park S, Choi EY, Choi D, Rim SJ & Chung N. (2008). Exaggerated blood pressure response to exercise is associated with augmented rise of angiotensin II during exercise. *Journal of the American College of Cardiology* **52,** 287-292.

Smith EE, Guyton AC, Manning RD & White RJ. (1976). Integrated mechanisms of cardiovascular response and control during exercise in the normal human. *Prog Cardiovasc Dis* **18,** 421-444.

Smith SA, Querry RG, Fadel PJ, Gallagher KM, Stromstad M, Ide K, Raven PB & Secher NH. (2003). Partial blockade of skeletal muscle somatosensory afferents attenuates baroreflex resetting during exercise in humans. *J Physiol* **551,** 1013-1021.

Stocker SD & Toney GM. (2005). Median preoptic neurones projecting to the hypothalamic paraventricular nucleus respond to osmotic, circulating Ang II and baroreceptor input in the rat. *The Journal of physiology* **568,** 599-615.

Tan PS, Killinger S, Horiuchi J & Dampney RA. (2007). Baroreceptor reflex modulation by circulating angiotensin II is mediated by AT1 receptors in the nucleus tractus solitarius. *American journal of physiology Regulatory, integrative and comparative physiology* **293,** R2267-2278.

Vallbo AB, Hagbarth KE & Wallin BG. (2004). Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol (1985)* **96,** 1262-1269.

Vianna LC, Deo SH, Jensen AK, Holwerda SW, Zimmerman MC & Fadel PJ. (2015). Impaired dynamic cerebral autoregulation at rest and during isometric exercise in type 2 diabetes patients. *American journal of physiology Heart and circulatory physiology* **308,** H681-687.

Victor RG, Seals DR & Mark AL. (1987). Differential control of heart rate and sympathetic nerve activity during dynamic exercise. Insight from intraneural recordings in humans. *J Clin Invest* **79,** 508-516.

Villapol S & Saavedra JM. (2015). Neuroprotective effects of angiotensin receptor blockers. *Am J Hypertens* **28,** 289-299.

Waki H, Gouraud SS, Maeda M & Paton JF. (2008). Specific inflammatory condition in nucleus tractus solitarii of the SHR: novel insight for neurogenic hypertension? *Auton Neurosci* **142,** 25-31.

Waki H, Gouraud SS, Maeda M, Raizada MK & Paton JF. (2011). Contributions of vascular inflammation in the brainstem for neurogenic hypertension. *Respiratory physiology & neurobiology* **178,** 422-428.

Wallin BG, Burke D & Gandevia S. (1994). Coupling between variations in strength and baroreflex latency of sympathetic discharges in human muscle nerves. *J Physiol* **474,** 331-338.

White DW, Shoemaker JK & Raven PB. (2015). Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci*.

Xue B, Pamidimukkala J & Hay M. (2005). Sex differences in the development of angiotensin II-induced hypertension in conscious mice. *Am J Physiol Heart Circ Physiol* **288,** H2177-2184.

Yamauchi M & Kimura H. (2008). Oxidative stress in obstructive sleep apnea: putative pathways to the cardiovascular complications. *Antioxid Redox Signal* **10,** 755-768.

Zucker IH, Wang W, Pliquett RU, Liu JL & Patel KP. (2001). The regulation of sympathetic outflow in heart failure. The roles of angiotensin II, nitric oxide, and exercise training. *Ann N Y Acad Sci* **940,** 431-443.

Figure Legends

Figure 1. Summary data of ESR amplitude response to an ACEi during baseline and two workloads of dynamic exercise (EX120) and (EX150). (N=8. Values are means ± SE); Placebo (PL), Captopril (CAP) and Perindopril (PER); † denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition.

Figure 2. (A) Systolic arterial pressures (SAP); (B) Diastolic arterial pressures (DAP) and (C) Mean arterial pressure (MAP) at rest and during dynamic leg exercise at workload HRs of 120 beats/min (E120) and workload HRs of 150 beats/min (E150). N=14 Values are means ± SE; Placebo (PL), Captopril (CAP) and Perindopril (PER); * denotes $P \leq 0.05$ compared to PL; † denotes $P \leq 0.05$ compared to CAP.

Figure 3. Muscle sympathetic activity (MSNA, arbitrary units (a.u.)) and arterial blood pressure (BP) tracings are shown for one representative subject during baseline (A) and both exercise workloads (B) EX120, (C) EX150 and under both conditions placebo (PL), captopril (CAP) and perindopril (PER).

Figure 4. (A) MSNA burst frequency (bursts/minute, top) N=7 and (B) percent change of MSNA burst frequency (bottom) at rest and during both exercise workloads (EX120) and (EX150). Values are means ± SE; Placebo (PL), Captopril (CAP) and Perindopril (PER);* denotes $P \leq 0.05$ compared to PL; † denotes $P \leq 0.05$ compared to CAP. ; ‡ denotes $P \leq 0.05$ compared to EX120 within condition.

Figure 5. Mean responses of blood pressure elected by perturbations of the carotid sinus baroreceptors at rest (A) and during both dynamic exercise workloads (B) EX120 and (C) EX150 Placebo (PL), Captopril (CAP) and Perindopril (PER); Arrows indicate the directional effect of

ACEi on baroreflex resetting compared with placebo. Data were fitted with the logistic function to represent the mean response across each condition. Values are means ± SE

Table 1. Hemodynamic responses to Angiotensin converting enzyme inhibitor and exercise

	Baseline			Exercise 120 (EX120)			Exercise 150 (EX150)		
Variable	PL	CAP	PER	PL	CAP	PER	PL	CAP	PER
HR, bpm	66 ± 2	62 ± 1	64 ± 2	121 ± 2#	119 ± 1#	117 ± 2#	151 ± 2#‡	144 ± 1#‡	145 ± 3#‡
SV, ml	80 ± 3	72 ± 5	70 ± 4	124 ± 5#	109 ± 6*#	105 ± 6*#	115 ± 5#‡	109 ± 6#	105 ± 5#
Q, l/min	5.3 ± .4	4.4 ± .3	4.5 ± .4	15.1 ± .2#	12.9. ± .1*#	12.2 ± .1*#	17.3 ± .3#‡	15.6 ± 1*#‡	15.2 ± 1*#‡

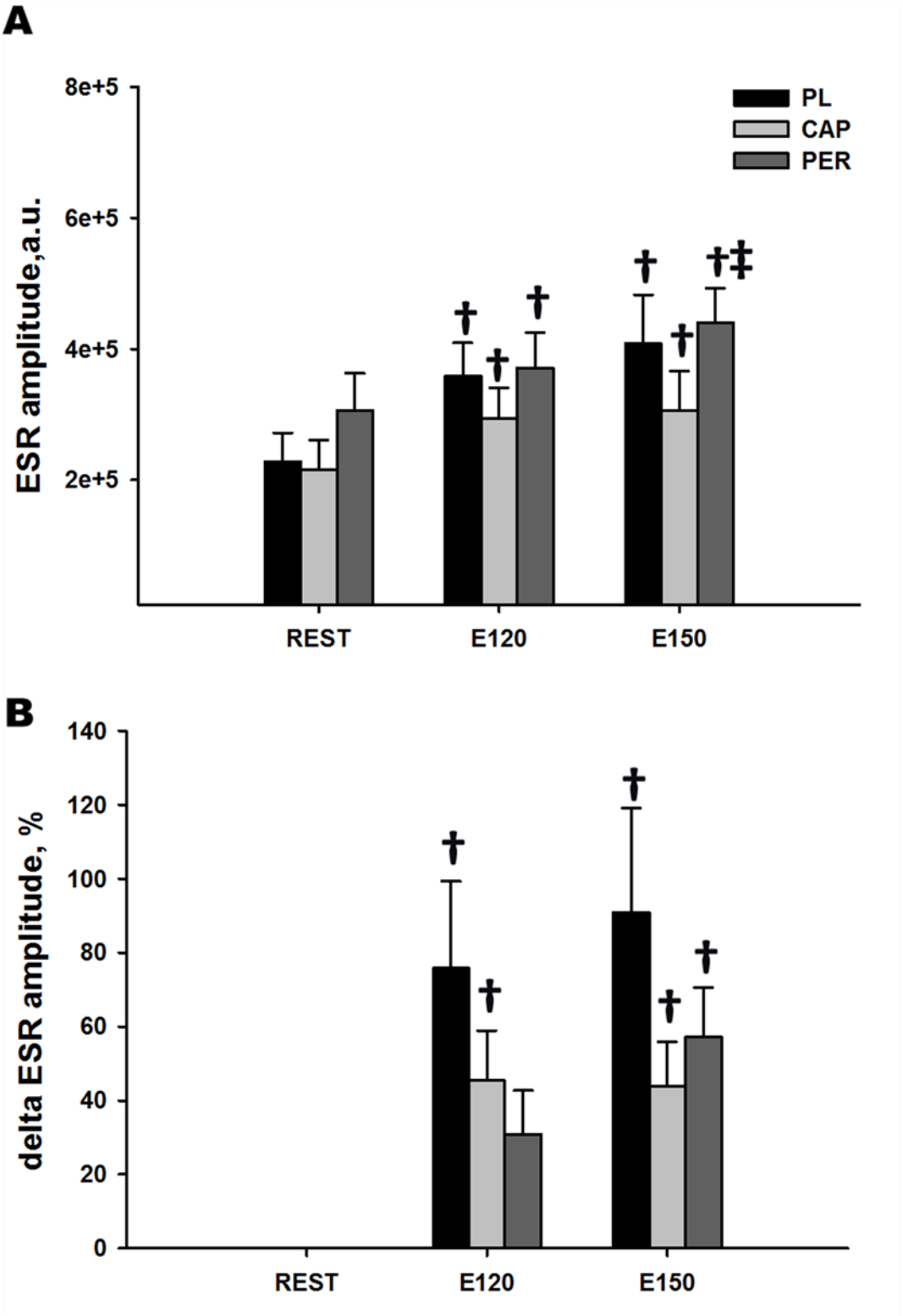
Values are means ± SE; n=14; Placebo (PL), Captopril (CAP) and Perindopril (PER); HR (heart rates); SV (stroke volume); Q (cardiac output); * denotes $P \leq 0.05$ compared to PL; † denotes $P \leq 0.05$ compared to CAP; # denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition

Table 2. Carotid-MAP baroreflex function curve parameter responses to Angiotensin converting enzyme inhibitor and exercise

	Baseline			Exercise 120 (E120)			**Exercise 150 (E150)**		
Variable	PL	CAP	PER	PL	CAP	PER	PL	CAP	PER
Response Range, mmHg	20 ± 2	23 ± 3	22 ± 2	18 ± 3	16 ± 2#	17 ± 1#	18 ± 2	19 ± 3	20 ± 1
Max Gain, mmHg/mmHg	-0.7 ± 0.1	-0.8 ± 0.1	-1.1 ± 0.1	-0.8 ± 0.1	-0.8 ± 0.1	-0.7 ± 0.1	-0.5 ± 0.1	-0.4 ± 0.1	-0.3 ± 0.1
Operating Point, mmHg	94 ± 2	87 ± 3	89 ± 2	109 ± 2#	102 ± 3#	**97 ± 3***	107 ± 3	103 ± 2	100 ± 2*
Threshold, mmHg	65 ± 7	54 ± 7	71 ± 6	90 ± 5#	89 ± 6#	**79 ± 2**	99 ± 7#	93 ± 5#	**69 ± 6*†**
Saturation, mmHg	111 ± 4	104 ± 5	101 ± 5	131 ± 3#	120 ± 5#	117 ± 4#	129 ± 10	140 ± 7	147 ± 7 ‡
Min. MAP Response, mmHg	85 ± 3	80 ± 2	81 ± 3	99 ± 3#	92 ± 2#	**88 ± 2***	96 ± 2	90 ± 4#	88 ± 2#

Values are means ± SE; n=9 E120; Placebo (PL), Captopril (CAP) and Perindopril (PER); response range equals maximum minus minimum mean arterial pressure (MAP) response. * denotes $P \leq 0.05$ compared to PL; † denotes $P \leq 0.05$ compared to CAP; # denotes $P \leq 0.05$ compared to baseline within condition; ‡ denotes $P \leq 0.05$ compared to EX120 within condition

Figure 1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6e+5
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ESR amplitude,a.u.
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REST
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delta ESR amplitude, %
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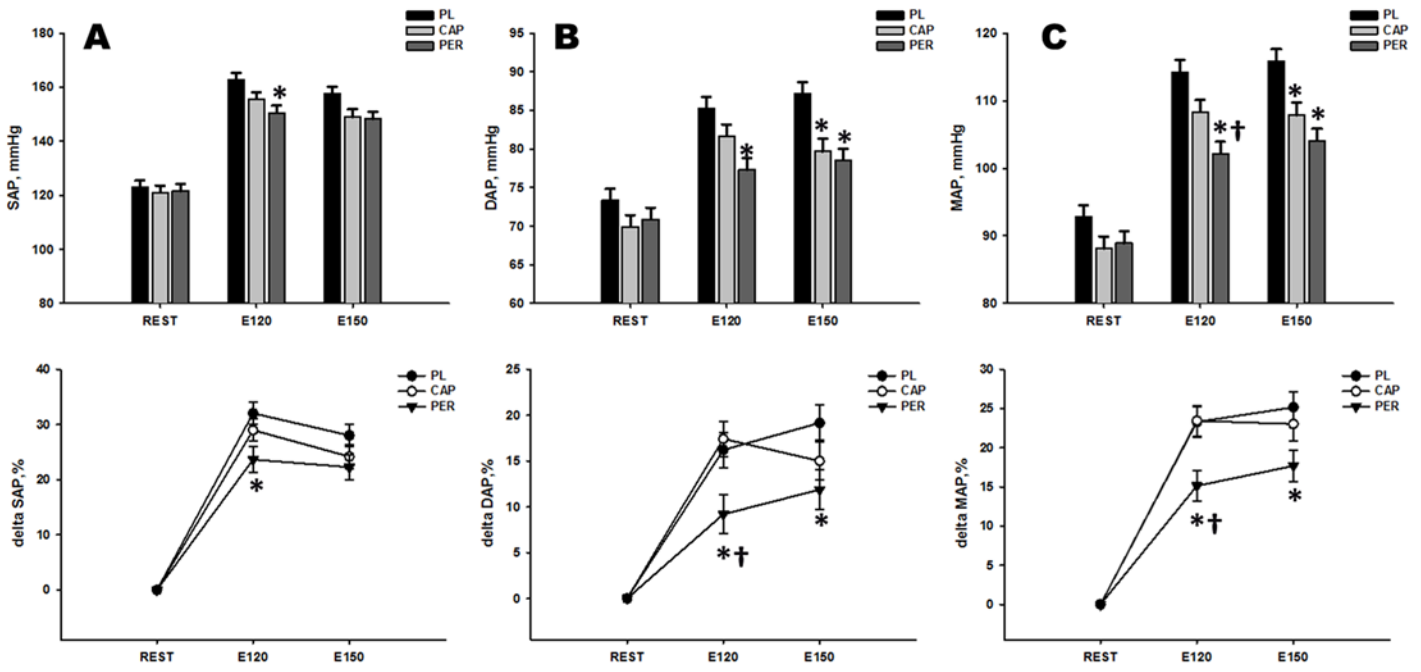
REST
E120
E150

Figure 2


A
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PL
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SAP, mmHg
DAP, mmHg
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delta MAP,%
REST
E120
E150

Figure 3

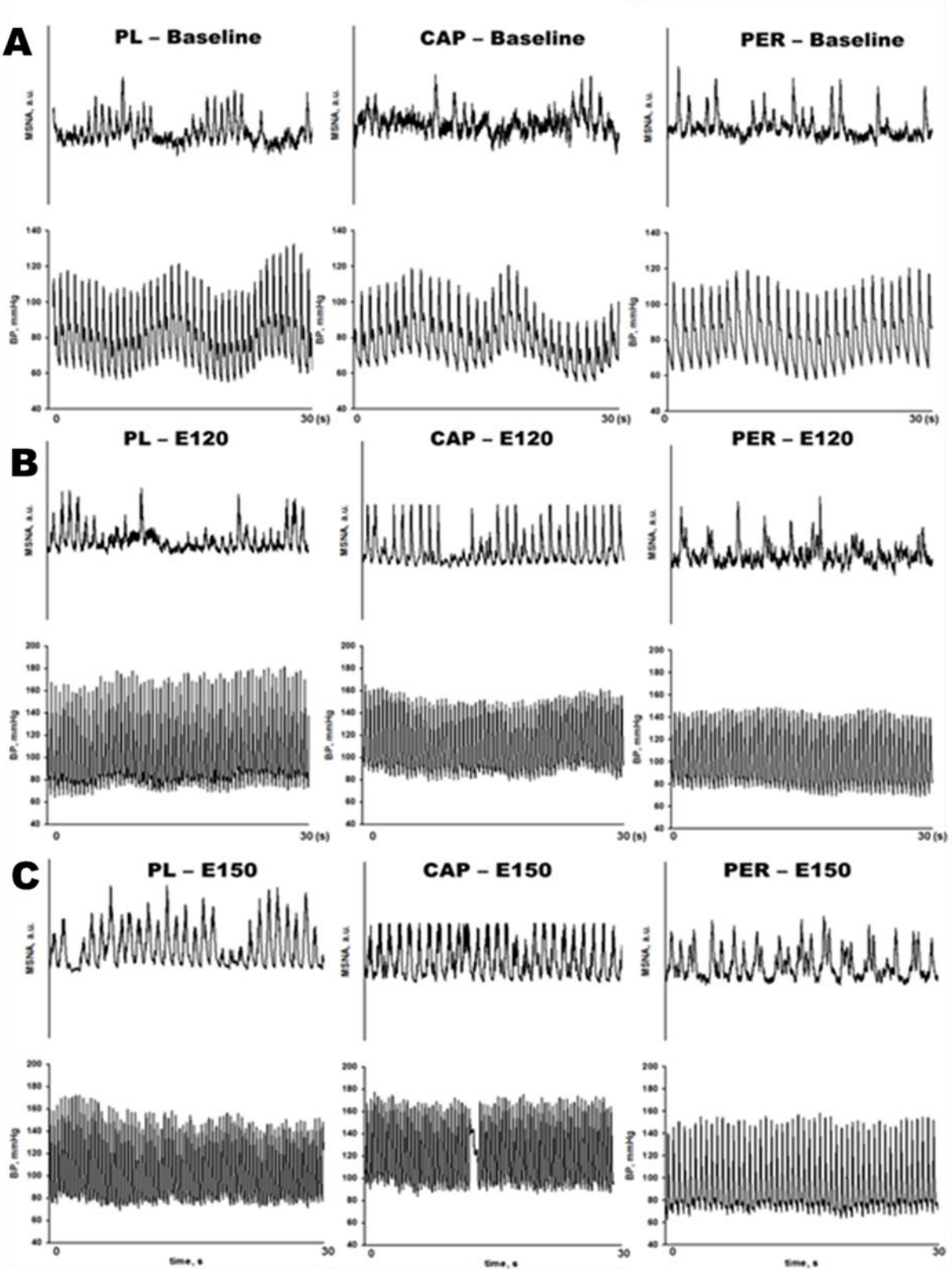
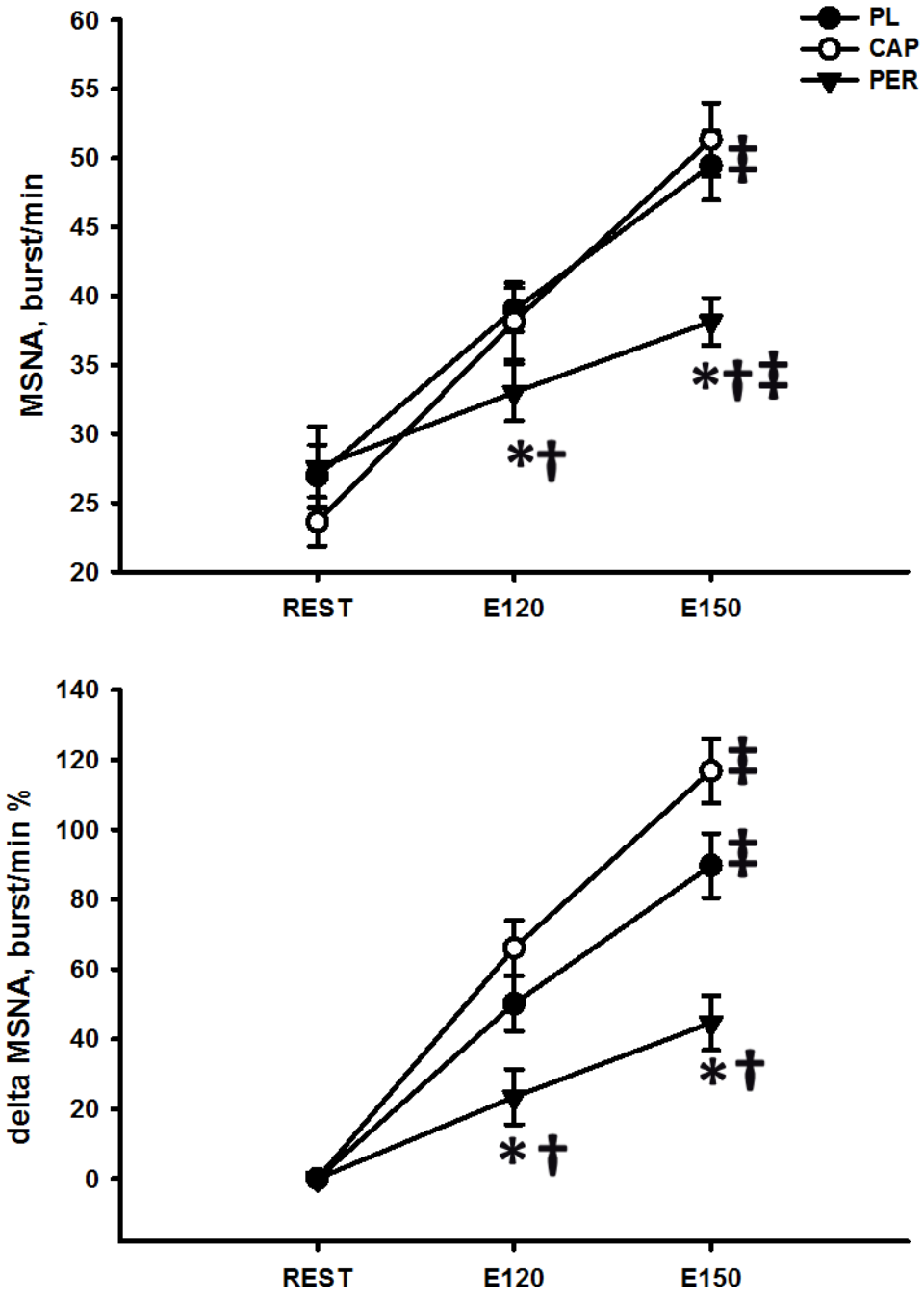

A
PL – Baseline
CAP – Baseline
PER – Baseline
MSNA, a.u.
BP, mmHg
0
30 (s)
B
PL – E120
CAP – E120
PER – E120
C
PL – E150
CAP – E150
PER – E150
time, s
30

Figure 4


PL
CAP
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MSNA, burst/min
60
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30
25
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REST
E120
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delta MSNA, burst/min %
140
120
100
80
60
40
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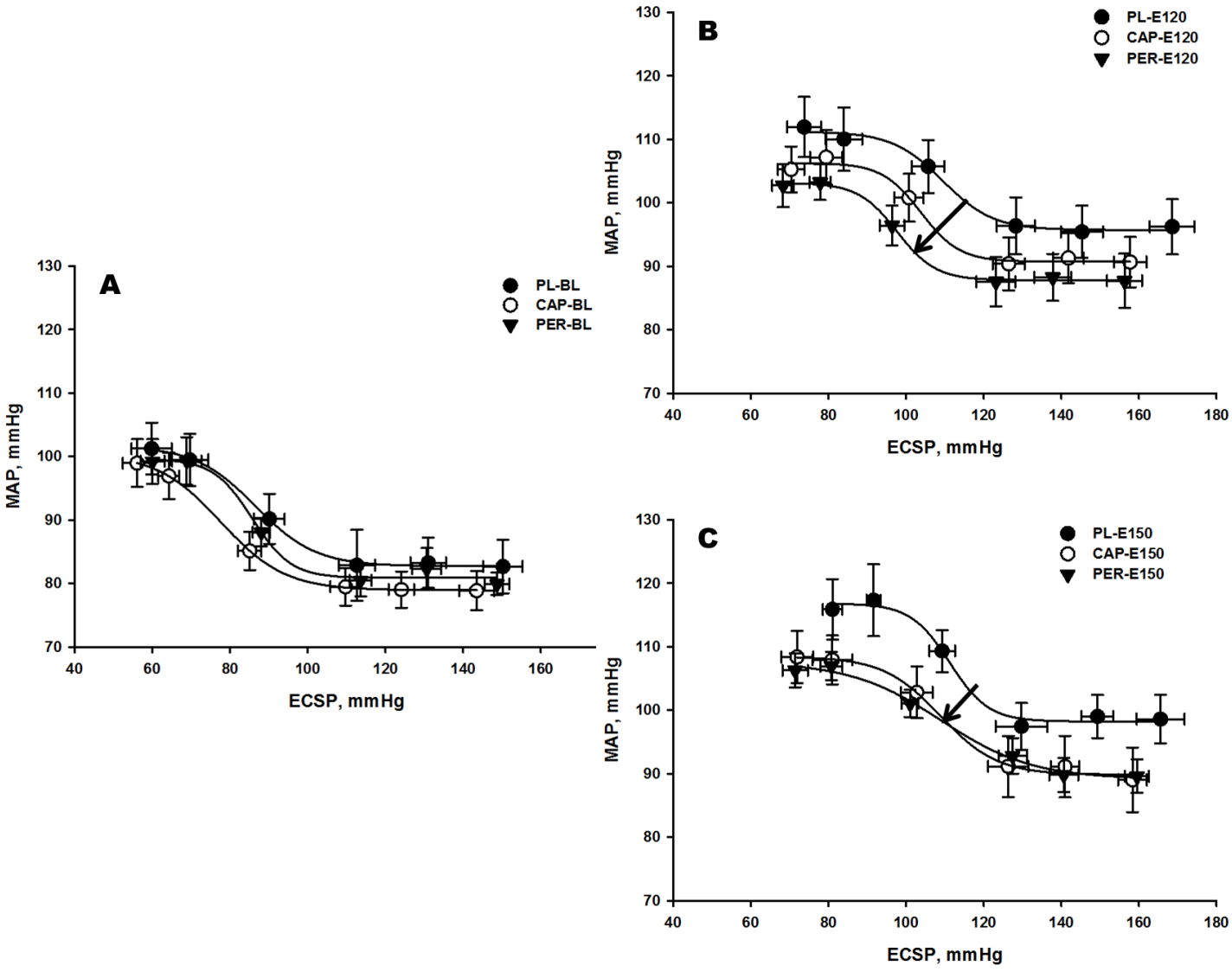
REST
E120
E150

Figure 5


A
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MAP, mmHg
ECSP, mmHg
B
PL-E120
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PER-E120
MAP, mmHg
ECSP, mmHg
C
PL-E150
CAP-E150
PER-E150
MAP, mmHg
ECSP, mmHg

CHAPTER IV

CONCLUSIONS

The results of the two investigations described within this dissertation identify that free radicals play a pivotal role in the exercise related resetting of the ABR control of blood pressure and MSNA in humans. Specifically, the objectives of these two studies were to determine the location (i.e. central or peripheral) of free radical production involved in ABR resetting.

The first investigation demonstrates that oral ingestion of a centrally and peripherally acting antioxidant cocktail resulted in a: i) marked reduction in plasma superoxide concentrations; ii) reduction in central muscle sympathetic nerve activity (MSNA) outflow; iii) downward and leftward resetting of the OP pressure of the ABR at rest and during exercise. Therefore, we established that free radicals play a pivotal role in the exercise related resetting of the ABR control of blood pressure and central MSNA outflow in humans.

The second investigation demonstrated that a single oral ingestion dose of a centrally and peripherally acting angiotensin converting enzyme inhibitor (ACEi), Perindopril (PER), resulted in an attenuation of the exercise induced resetting of the MAP-OP and threshold pressures when compared to PL and CAP. These findings indicate that central ANG II production plays a pivotal role in the full expression of the exercise related rightward and upward resetting of the arterial baroreflex OP pressure and the reflex control of central MSNA outflow.

The findings from these investigations, not only advance our understanding regarding the physiological mechanisms governing reflex cardiovascular control during exercise in healthy humans but, also, provides insight into the patho-physiological mechanisms of impaired autonomic regulation of cardiovascular responses such as exercise induced hypertension (EIHt).

CHAPTER V

SUGGESTIONS FOR FUTURE RESEARCH

Although the research presented in this dissertation provides several new findings regarding the exercise intensity related rightward and upward resetting of the arterial baroreflex OP pressure and the reflex control of central sympathetic outflow several questions remain unanswered.

I. Ang II stimulation of NAD(P)H oxidase–dependent generation of superoxide results from the activation of the Ang II binding to Angiotensin -1 receptors (AT_1 R).In order to test the hypothesis that free radical mediated ABR-MSNA resetting during exercise is mediated by Ang II binding to AT_1Rs located within the brain, the following experiments need to be performed comparing the acute ABR-MSNA resetting from rest to exercise: i) without (placebo) and , ii) with pharmacological inhibition of the AT_1R using effective dose equivalents of Candesartan, a highly lipid soluble, AT_1R blocker that readily crosses the BBB, and Valsartan, a non-lipid soluble AT_1R blocker that does not cross the BBB. I would anticipate that the selective **AT_1** receptor blockade of the central and peripheral AT_1Rs of ANG II production would reduce sympathetic nerve activity and arterial baroreflex resetting more during exercise compared with peripheral blockade alone.

II. Further experiments may include the measurement of arterio-venous differences in free radicals, catecholamines, nitrites and nitrates and Ang II across the brain and the systemic circulation in healthy subjects at rest and during exercise. Completion of these set of experiments will directly identify the global role of FRs, and more specifically, the central or peripheral role of Ang II mediated FR production in physiologic ABR resetting

during exercise. Subsequent experiments involving EIHt patients and using similar experimental designs will provide insight into the underlying pathophysiologic signaling mechanisms involved with EIHt.