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The importance of physical activity is well established as a means to maintain good health. However, under certain conditions and in some individuals, heavy exercise leads to catastrophic failure of the cardiovascular system. This is especially true during early recovery from exercise. This may be due in part to an improper response of the autonomic nervous system; that is, an imbalance of the sympathetic and parasympathetic nervous systems. The purpose of the investigations presented in this dissertation was to: i) re-evaluate the commonly accepted model of autonomic influence on control of heart rate during exercise; ii) study the effects of posture on recovery from heavy exercise; and iii) determine the effect of muscle pump activity on cardiorespiratory control of the cardiovascular system during the transition from active to inactive recovery following heavy dynamic two legged cycling. In the first investigation we examined previously reported and newly collected data and determined a fine balance exists between the sympathetic and parasympathetic nervous systems throughout all intensities of exercise. Our conclusions led to the development of a new model of autonomic balance during exercise. In the second investigation we concluded that unloading of the cardiopulmonary baroreceptors by upright posture significantly increases baroreflex control of heart rate during rest and during recovery from heavy dynamic leg cycling exercise. We also show that steady-state blood pressure and the baroreflex control of blood pressure is not significantly different based on orthostatic posture before or after

exercise. In the third investigation we concluded that loading of the cardiopulmonary baroreceptors by muscle pump activity during active recovery from heavy exercise diminishes the respiratory induced changes in cardiovascular function observed during inactive recovery. Overall, these investigations highlight the importance of the autonomic nervous system during exercise and during recovery from heavy exercise. Collectively, these conclusions should influence the decision making process regarding mode of recovery from heavy exercise, especially in an “at risk” population, because recovery is the time when most adverse events take place.

AUTONOMIC NERVOUS CONTROL OF CARDIOVASCULAR FUNCTION DURING AND FOLLOWING PROLONGED EXERCISE IN HUMANS

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DISSERTATION

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By

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AWARDS

Dept of Integrative Physiology, GSBS 2012

-Outstanding Graduate Student in Integrative Physiology for 2011-2012

Research Appreciation Day 2012

-Cardiovascular Research Institute *Graduate Student Research Achievement Award* $300

Texas Chapter of the American College of Sports Medicine Annual Meeting 2012

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LIST OF ABBREVIATIONS

ABR	Arterial Baroreflex
ABP	Arterial Blood Pressure
ABs	Arterial Baroreceptors
ANOVA	Analysis of Variance
ANS	Autonomic Nervous system
BP	Blood Pressure
CBR	Carotid Baroreflex
CBV	Central Blood Volume
CC	Central Command
CPBR	Cardiopulmonary Baroreflex
CVLM	Caudal Ventrolateral Medulla
CVP	Central Venous Pressure
DAP	Diastolic Arterial Pressure
ECG	Electrocardiogram
ECSP	Estimated Carotid Sinus Pressure
EIHt	Exercise Induced Hypertension
EPR	Exercise Pressor Reflex

HR	Heart Rate
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
NP	Neck Pressure
NS	Neck Suction
NTS	Nucleus Tractus Solitarius
PEMI	Post-Exercise Muscle Ischemia
PSNA	Parasympathetic Nerve Activity
PSNS	Parasympathetic Nervous System
Q	Cardiac Output
RPE	Rating of Perception of Effort
RVLM	Rostral Ventrolateral Medulla
SNA	Sympathetic Nerve Activity
SEM	Standard Error of the Means
SR	Semi-Recumbent
SV	Stroke Volume
UR	Upright

CHAPTER I

Introduction to the Study

A question which has yet to be answered by integrative physiologists is, “How are the cardiovascular changes during dynamic exercise neurally modulated?” Many published reviews outline the current body of research in the field (Rowell, 1974, 1980; Ludbrook, 1983; Mitchell, 1990; Rowell & O'Leary, 1990; Rowell, 1991; Zucker & Gilmore, 1991; Mitchell, 2012; Raven, 2012), but to date, the exact answer to this fundamental question has not been determined. This chapter will identify the need for continued research and development of unique approaches to the study of cardiovascular regulation during exercise.

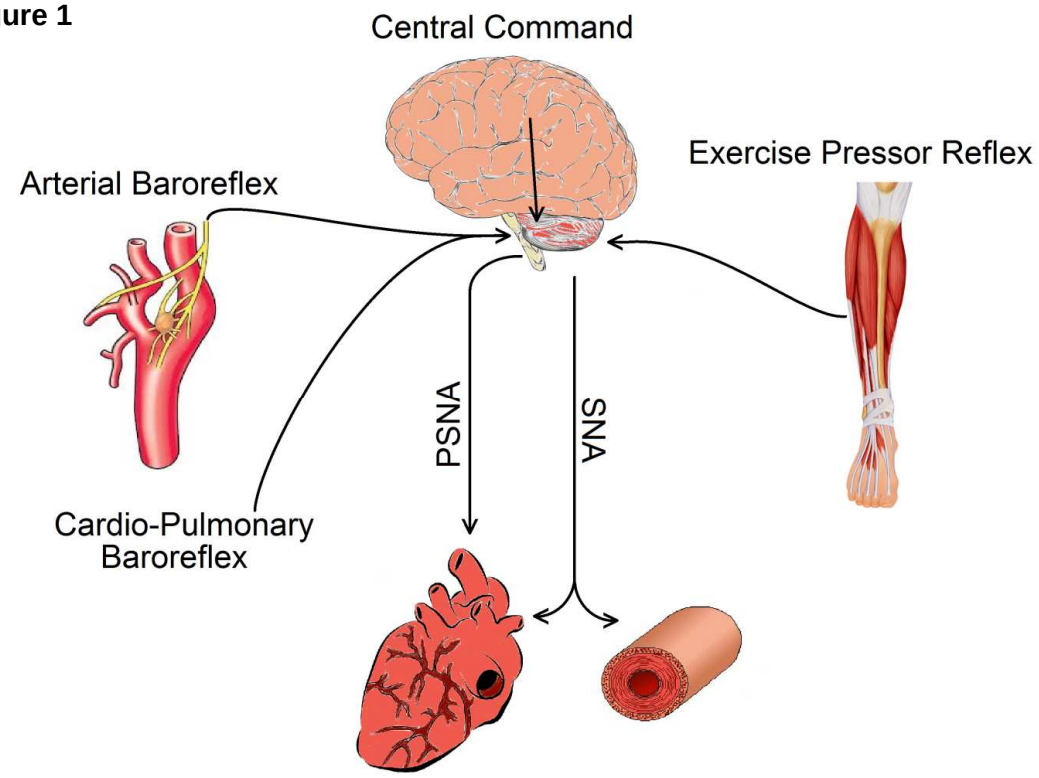
In 1841 Volkmann (Volkmann, 1841) suggested the presence of a latent afferent neural signal emanating from the contracting skeletal muscle that increased heart rate (HR). This finding was followed by Johannson in 1893 (Johansson, 1893) in which he identified a simultaneous increase in the rabbit’s HR at the onset of muscle contraction indicating an efferent neural signal arising from within the brain. However, it was not until the early work of Krogh and Lindhard using human subjects, measuring HR and cardiac output (Q), during 1913 and 1917 (Krogh & Lindhard, 1913, 1917), along with the work of Alam and Smirk (Alam & Smirk, 1937) measuring auscultatory blood pressures during exercise and with post-exercise muscle ischemia (PEMI), that two distinct major neural control mechanisms were identified. The two neural mechanisms are currently named: i.) ‘Central Command’ (CC), which is identified as

neural signals starting in the motor cortex feeding forward via neural pathways, not fully identified, to integrate with peripheral neural inputs within the nucleus tractus solitarius (NTS) to provide autonomic efferent signals to the heart and blood vessels (Mitchell, 1985, 1990; Raven, 2012); and ii.) the 'Exercise Pressor Reflex' (EPR) which involves afferent neural signals arising from the exercising skeletal muscle (Mitchell *et al.*, 1983; Secher & Amann, 2012), that integrate within the NTS with CC and other peripheral inputs resulting in efferent neural signals to the heart and the peripheral blood vessels (Raven, 2012). The EPR is stimulated by an error signal resulting from the buildup of metabolites within the active muscle and also by contractions of the musculature, metabo- and mechano-reflexes, respectively (Kaufman *et al.*, 1983; Mitchell *et al.*, 1983; Kaufman *et al.*, 1984).

Additionally, two peripheral neural inputs to the NTS have been identified and confirmed to contribute to the exercise-induced cardiovascular responses. Both are feedback mechanisms, one that monitors arterial blood pressure (ABP) and the other that monitors central blood volume (CBV), or cardiac filling volume (Fadel & Raven, 2012). On the high pressure side of the cardiovascular system the aortic and carotid arterial baroreceptors (ABs) are anatomically positioned to sense both the arterial pressure of the systemic circulation and the cerebral circulation. Increases in ABP increase the transmural pressure of the ABs and activate the mechanoreceptors within the wall of the ABs sending an increased afferent signal to the NTS. This afferent signal is compared to a centrally established "set point" within the cardiovascular center and results in reflex neural efferent signals to decrease Q and vasodilate the peripheral blood vessels. Decreases in ABP eliciting a decrease in the transmural pressure of the ABs result in reduced afferent signals to the NTS and reflex increases in Q and peripheral vasoconstriction (Heesch, 1999).

On the low pressure side, the cardiopulmonary baroreflex (CPBR) has been shown to regulate changes in blood pressure and sympathetic nerve activity (Fadel & Raven, 2012). The CPBR may act as a brake on the CC and EPR induced increases in the operating point of the arterial baroreflex. Increases in central blood volume whether from orthostatic changes (Smith *et al.*, 1987), lower body positive pressure (Shi *et al.*, 1993) or muscle pump activity (Sprangers *et al.*, 1991) decrease the response range of the arterial baroreflex and also decreases the magnitude of resetting of the operating point for a given workrate; e.g. pedal frequency changes (Ogoh *et al.*, 2007) or arm+leg cycling (Volianitis *et al.*, 2004). All of these inputs must be integrated and the output modulated during exercise to allow for the appropriate hemodynamic changes to occur. However, the fundamental question remains as to "what influences the control of hemodynamics during exercise?"

Figure 1



Crucial to controlling cardiac output and systemic blood pressure is the autonomic nervous system. A fine balance between sympathetic nervous activity (SNA) and parasympathetic nervous activity (PSNA) is essential for maintaining hemodynamic homeostasis. This balance needs to adapt to the present demands of the body; e.g. fear, hemorrhage, exercise. Each of these conditions elicits an increase in SNA and a decrease in PSNA which collectively attempt to increase cardiac output, venous return and blood pressure. In the case of fear, an emotional limbic response drives SNA and causes the hemodynamic response. With hemorrhage, a decrease in the afferent firing of the baroreflexes, arterial and cardiopulmonary, and a lack of flow through the kidneys increases SNA. But, recreational exercise does not involve fear or major loss of blood, and actually depending on the mode of exercise, may increase afferent firing from the baroreceptors due to muscle pump activity, as in dynamic leg cycling increasing venous return, CBV, Q and BP. This increase in afferent firing should logically cause a decrease in SNA to the heart and blood vessels. 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 & Waki, 2009).

Recent reports have shown that low workload dynamic cycling causes decreases in SNA, but in contrast, increased workloads elicit increased SNA during dynamic leg cycling exercise (Saito *et al.*, 1993; Ichinose *et al.*, 2008). This follows the concept of baroreflex resetting, in which the reflexive responses establish an operating point to reflect the increased ‘set point’.

Yet to be elucidated in the literature are the neural cardiovascular responses during early recovery from exercise, when the baroreflexes should be returning to resting set points.

Specifically, recovery HR following prolonged dynamic exercise is a topic not fully clarified in the literature (Coote, 2010). It is mostly agreed upon that an increase in parasympathetic activity following the cessation of exercise contributes to most of the initial decrease in HR followed by the slower decrease in sympatho-adrenal activity eventually ending in HR returning to resting values (Kannankeril & Goldberger, 2002; Kannankeril *et al.*, 2004; Pierpont & Voth, 2004; Coote, 2010), though conflicting reports state more influence of sympathetic withdrawal in early recovery (Savin *et al.*, 1982). The contribution of afferent neural signals has not been fully explored in humans. This is important because measuring recovery HR has become more evident to clinicians in recent years as a predictor of future pathogenesis (Cole *et al.*, 1999; Buch *et al.*, 2002).

Exercise induced hypertension (EIHt) has also been correlated with the future onset of essential hypertension and is suggested as a means of early detection of essential hypertension (Manolio *et al.*, 1994; Matthews *et al.*, 1998; Miyai *et al.*, 2002; Pescatello *et al.*, 2004). Mayai et al. (Miyai *et al.*, 2002) found that a disproportionate BP response to exercise of a resting normotensive individual indicated a threefold higher risk of developing hypertension within 5 years and Manolio et al. (Manolio *et al.*, 1994) reported that among 18 to 30 year olds, an exaggerated BP response to exercise held a 1.7x greater likelihood of developing hypertension within 5 years. Retrospective studies by Matthews et al. (Matthews *et al.*, 1998) of graded exercise data collected between 1971 and 1982 identified in a 1986 follow up questionnaire that those individuals that reported physician diagnosed hypertension were more likely to have had EIHt in the graded exercise test. Another implicated predictor of future hypertension is the maintenance of elevated blood pressure well after the end of exercise (Davidoff *et al.*, 1982; Singh *et al.*, 1999). The American College of Sports Medicine identifies that an abnormal or

exaggerated response of BP to exercise contributes to the prediction of the future onset of hypertension (Pescatello *et al.*, 2004). However, the exact mechanism for exercise-induced hypertension remains obscure (Ogawa *et al.*, 2009).

Due to the questions raised and the limited information in the literature, this dissertation is focused on the autonomic control of the cardiovascular system during exercise; specifically on the influence of sympathetic nerve activity and control during early recovery.

Related literature

Anatomy of the Autonomic Nervous System

Arterial baroreceptors are mechano-receptors located in the carotid sinuses and aortic arch (Kirchheim, 1976). They are the anatomical starting point of the negative feedback system for control of arterial pressure. The increases in pressure within the carotid sinuses or aortic arch increase afferent firing via cranial nerve IX (CNIX) and cranial nerve X (CNX), respectively. The increase in afferent nerve traffic is integrated within the nucleus tractus solitarius (Korner, 1971; Calaresu *et al.*, 1975). Excitatory interneural signals are then sent to the nucleus ambiguous (NA) and the caudal ventrolateral medulla (CVLM). Parasympathetic efferent neural signals exit the NA and travel through CNX and synapse with post ganglionic fibers within the ganglionated plexuses of the heart (Armour *et al.*, 1998). Signals exciting the CVLM cause the release of gamma aminobutyric acid (GABA) onto the rostral ventrolateral medulla (RVLM) causing inhibition of sympathetic outflow. Sympathetic pre-ganglionic fibers project from the RVLM through the intermediallateral column of the spine and synapse with the post ganglionic neurons at sympathetic ganglia (Potts, 2002).

Aortic and Carotid Baroreflexes

The arterial baroreflex (ABR) was originally described by Marey in 1863 as an inverse relationship in heart rate to changes in blood pressure (Marey, 1863). The identification of this inverse relationship resulted in one and a half centuries of investigations into the anatomy, neural control and function of the arterial baroreflex (Sheehan *et al.*, 1941; Heymans, 1958; Mancia & Mark, 1983). Increases and decreases in blood pressure cause conformational changes in the carotid and aortic baroreceptors engaging and disengaging the vessel wall's mechanoreceptors, respectively, producing the afferent signals. These afferent neural signals integrate at the NTS with CC, EPR and CPBR afferent inputs (Aicher & Randich, 1990) and after integration and comparison with the central 'set point' results in appropriate reflexive autonomic efferent neural activity. An increase in carotid sinus or aortic baroreceptor transmural pressure causes an increase in afferent neural activity resulting in an increase in PSNA and a decrease in SNA to the heart. The overall result is a decrease in cardiac output due to decreased heart rate and also a decrease in systemic vascular resistance by reducing sympathetically mediated vasoconstriction of the peripheral blood vessels, disfacilitation of the vascular tone. Alternatively, a decrease in afferent firing from the mechanoreceptors of the carotid and aortic baroreceptors caused by a decrease in blood pressure causes an increased cardiac output and increased vascular resistance. The arterial baroreflex is the body's first line of defense against changes in systemic pressure due to orthostasis, hemorrhage, exercise and other pressure altering stimuli, but the arterial baroreflex does not operate alone.

Cardiopulmonary baroreflex

The CPBR has been shown to regulate changes in blood pressure and SNA (Shi *et al.*, 1993; Charkoudian *et al.*, 2004; Fadel & Raven, 2012). The CPBR is a slower responding system as opposed to the beat-to-beat responses of the ABR. The CP baroreceptors are located in

the right heart, the great veins and also in the blood vessels of the lungs (Mark & Mancia, 1983; Hainsworth, 1991; Ray & Saito, 1999). It is generally accepted that the CPBR adapts the autonomic control of hemodynamics to the steady state CBV within the great veins and lungs. Central blood volume, whether from orthostatic changes (Smith *et al.*, 1987), lower body positive pressure (Shi *et al.*, 1993) or muscle pump activity (Sprangers *et al.*, 1991) decreases the response range of the arterial baroreflex and also decreases the magnitude of resetting of the operating point for a given exercise work rate; e.g. pedal frequency changes (Ogoh *et al.*, 2007) or arm/leg cycling (Volianitis *et al.*, 2004). The combination of the CPBR and ABR make up the negative feedback loop of blood pressure control.

Central Command

Originally proposed in 1886, cortical irradiations projecting from the motor cortex area of the brain into the cardiovascular control centers enabled simultaneous parallel activation of the cardiovascular system with muscular activation, resulting in increases in blood pressure and heart rate at the onset of exercise (Zuntz & Geppert, 1886; Johansson, 1893; Krogh & Lindhard, 1913). This has since been termed "central command" (Goodwin *et al.*, 1972). The magnitude of the effect of central command is largely due to the perception of effort of the individual performing the task (Mitchell, 1990), therefore, more perceived effort equals greater increases in blood pressure and MSNA. Central command is the primary feed-forward mechanism in cardiovascular control during exercise and is thought to be involved in anticipatory stimulation of the cardiovascular system before exercise onset (Williamson, 2010).

Exercise Pressor Reflex

In 1937 Alam and Smirk reported that muscle ischemia produced by inflating a blood pressure (BP) cuff around the upper arm and reducing arterial blood flow into the forearm increased the blood pressure response to handgrip exercise and that the blood pressure remained elevated post-exercise until the BP cuff was deflated and the muscle ischemia removed (Alam & Smirk, 1937). Similar results have been reported with dynamic leg cycling (Rowell *et al.*, 1976). Subsequent investigations (Mitchell *et al.*, 1983; Secher & Amann, 2012) repeated these findings and recorded reflex heart rate and blood pressure responses identifying the presence of an exercise pressor reflex (EPR). Two sensory mechanisms have been determined to be involved in the EPR, one which is stimulated by an error signal resulting from the buildup of metabolites within the active muscle and the other by contractions of the musculature, metabo- and mechano-reflexes, respectively (Kaufman *et al.*, 1983; Mitchell *et al.*, 1983; Kaufman *et al.*, 1984; Kaufman, 2012). An increase in MSNA has also been identified that the mediator of this blood pressure raising reflex (Mark *et al.*, 1985). The increase in MSNA and the subsequent elevated blood pressure are returned to baseline after the release of the ischemia. Importantly, there is no maintenance of elevated HR after the cessation of exercise (Alam & Smirk, 1938) with muscle ischemia. O'Leary (O'Leary, 1993) proposed that "vagal withdrawal" was terminated at the end of exercise but Fisher (Fisher *et al.*, 2010) reported that even with parasympathetic blockade, exercising heart rate is not maintained after exercise during muscle ischemia. Potts proposed that the exercise pressor reflex afferents synapsed directly on the RVLM (Potts, 2006) which would explain the lack of heart rate maintenance post-exercise. This has since been substantiated by Kaufman (Kaufman, 2012) in animals. The EPR induced increase in central sympathetic outflow is important for the shunting of blood away from the non-working muscle

and splanchnic circulation. The EPR plays an important role in providing negative feedback to the NTS in modulating the hemodynamic control of the circulation during exercise.

Integration of baroreflex control during exercise

At the onset of dynamic exercise, there is a brief period of time during which diastolic arterial pressure (DAP) and MSNA decrease, probably as a result of the muscle pump increasing venous return and loading the CPBR (Shi *et al.*, 1993; Charkoudian *et al.*, 2004). After this brief decrease in DAP the blood pressure quickly elevates and there is (using logic applicable to resting conditions) an atypical tachycardia and subsequent increase in cardiac output. This is the phenomenon of baroreflex resetting. It was long thought that the baroreflexes were "turned off" during exercise because of the lack of the reflex bradycardia associated with the resting baroreflex (Pickering *et al.*, 1972; Mancia *et al.*, 1978). This was difficult to corroborate or disprove because common techniques used to assess baroreflex function usually measured heart rate responses during drug induced changes in blood pressure (Smyth *et al.*, 1969). It has since been discovered that the reason for the lack of a heart rate change during high intensities of exercise when a hypotensive drug is given is that the operating point of the arterial baroreflex has moved to a point of lesser gain and, therefore, does not respond with the same magnitude of tachycardia as in resting conditions. With the utilization of the neck pressure/ neck suction (NP/NS) technique during progressive increases in steady-state dynamic exercise intensity, Potts et al. (Potts *et al.*, 1993; Fadel & Raven, 2012) found that both heart rate and blood pressure reflexes are reset to higher operating point pressures. The reset operating point pressures are linearly associated with increases in exercise intensity. In addition, the maximum baroreflex sensitivity (max gain) carotid-vasomotor reflex remained unchanged (Potts *et al.*, 1993).

Other techniques of analyzing baroreflex sensitivity consist of computer data acquisition of beat-to-beat HR and ABP to determine a spontaneous baroreflex gain from the linear relationship between three heart beats and blood pressures (Bertinieri *et al.*, 1985; Iellamo *et al.*, 1997) and/or the linear dynamic analysis of the transfer function between the power spectral densities of the HRs and BPs (Zhang *et al.*, 2001).These analyses were examined by Ogoh et al. (Ogoh *et al.*, 2005) and compared to the NP/NS data obtained at steady-state HRs at rest, and at exercise HRs of 90, 120 and 150 beats/min. The results of these comparisons identified that the spontaneous baroreflex and transfer function gain analyses only identified the gains of the HR reflex at the operating point pressure and confirmed that the carotid-HR reflex was relocated to a point of lesser gain on the carotid baroreflex function curve as the exercise intensity increased.

All four of the neural mechanisms (ABR, CPBR, EPR, and CC) work concurrently during exercise to regulate sympathetic and parasympathetic efferent nerve activity to the heart and blood vessels (Fadel & Raven, 2012). Integration of the neural signals within the NTS has to be modified to enable changes in reflex function. However, the signaling mechanism involved in the intensity related resetting of the arterial baroreflex is unknown. Furthermore, cases have been made that indicate that resetting of the arterial baroreceptors and/or resetting of the neural arc (NTS-CVLM-RVLM) in the hindbrain may occur. Rowell suggested that with sustained increases in pressure, the visoelastic fibers in the carotid sinus would eventually relax causing the receptor to reset (Rowell, 1993). Rowell also suggested that stretch activated sodium channels could depolarize the receptors which would lower afferent firing at elevated pressures and reset the baroreflex (Rowell, 1993). However, in contrast to a peripheral resetting at the baroreceptors, Tan et al. (Tan *et al.*, 1989) reported a central mechanism of resetting in conscious dogs where one isolated carotid sinus was trained and the contralateral was stimulated. The result

was a resetting of the non-stimulus trained baroreceptor. This finding, because at the onset of dynamic exercise HR and BP increase in parallel, supports the hypothesis that the exercise related arterial baroreflex resetting has a central origin.

Our model proposes that the interaction of all the afferent inputs converging at the NTS enables resetting. Information from the ABRs and the CPBR may act as a brake on the CC and EPR induced increases in the operating point of the arterial baroreflex, thereby, limiting the reflex curve from resetting to a hypertensive point.

Control during Recovery

An active area of research is the neural control of cardiovascular function during recovery from exercise. Prior to the 1950s, recovery HRs following many types of standardized exercise stress tests were used to provide assessments of cardiovascular fitness. Early examples of using recovery HRs for estimating an individual's cardiovascular fitness was the Harvard Step Test (Gallagher & Brouha, 1943) and another for estimating clinical risk of heart disease was the Master's two-step (Master, 1950). Subsequently, with the introduction of exercise stress testing that utilized bicycle ergometers and motor driven treadmills and continuous electrocardiogram (ECG) recordings during the testing, the fitness predictions and cardiovascular risk estimates from recovery HRs had fallen into abeyance. More recently, the need for an understanding of cardiovascular control during recovery has been rejuvenated, due in part to the realization that measuring recovery HR has become more evident to clinicians in recent years as a predictor of future pathogenesis (Cole *et al.*, 1999; Buch *et al.*, 2002).

The neural regulatory mechanisms involved at the onset of exercise and during HR recovery have recently been reviewed by Coote (Coote, 2010). Our understanding of the neural

mechanisms involved in downward resetting of the arterial baroreflex during reduced workload, cessation of work and recovery from exercise is, however, still incomplete. One investigation into the changes in HR and MAP that occur during recovery identified that the mode of recovery (inactive, active or passive) plays a significant role in the maintenance of cardiac output and mean arterial pressure following short duration (3min) upright submaximal cycling exercise (Carter *et al.,* 1999). Furthermore, the authors indicated that their data suggests that central command does not appear to play an important role in maintaining blood pressure and cardiac output during recovery, but probably does influence the recovery heart rate (Carter *et al.*, 1999). Halliwill *et al*. (Halliwill *et al.,* 2013) summarized their work since 2001 in which the group investigated potential mechanisms underlying post-exercise hypotension during 60 mins of supine recovery after performing a standard, upright, 60% peak oxygen uptake (VO_2peak) exercise. Their cumulative findings identified that skeletal muscle afferents are involved in the arterial baroreflex post-exercise downward resetting by affecting discrete receptor changes within the NTS. However, there remain conflicting reports on parasympathetic nervous system function during early recovery from prolonged exercise in humans (Savin *et al.*, 1982; Kannankeril & Goldberger, 2002; Kannankeril *et al.*, 2004; Pierpont & Voth, 2004; Coote, 2010). A reduction in MSNA at 60 minutes post-exercise in borderline hypertensive men has been identified (Floras *et al.*, 1989), along with similar reports from Halliwill *et al*. (Halliwill *et al.*, 1996) at 60 minutes into supine recovery.

To date, there have been no direct neural recordings demonstrating the characteristics of MSNA in healthy humans during early recovery from prolonged leg cycling exercise. Accentuated antagonistic interactions of the parasympathetic and sympathetic branches of the ANS are known to exist (Levy, 1971), which may be modified during recovery. The limited

information in the literature and the advancement of data collection and analysis techniques has lead to the current set of investigations.

HYPOTHESIS:

We hypothesize that the exercise-induced changes in cardiopulmonary receptor loading contribute significantly to post exercise cardiovascular control. We expect that posture and muscle pump activity, increasing venous return and central blood volume, will decrease autonomic control of the cardiovascular system.

Specific aims

Understanding the mechanisms that regulate cardiovascular function in health is paramount to understanding pathological mechanisms. Tan et al. demonstrated that unilateral carotid sinus pressure training in conscious aortic denervated dogs caused contralateral ABR resetting, indicating that ABR resetting occurs centrally (Tan *et al.*, 1989). We have confirmed in humans that a central mechanism is involved in ABR resetting during exercise (Fadel & Raven, 2012). But what is unknown is the influence of peripheral inputs on modulating this central mechanism.

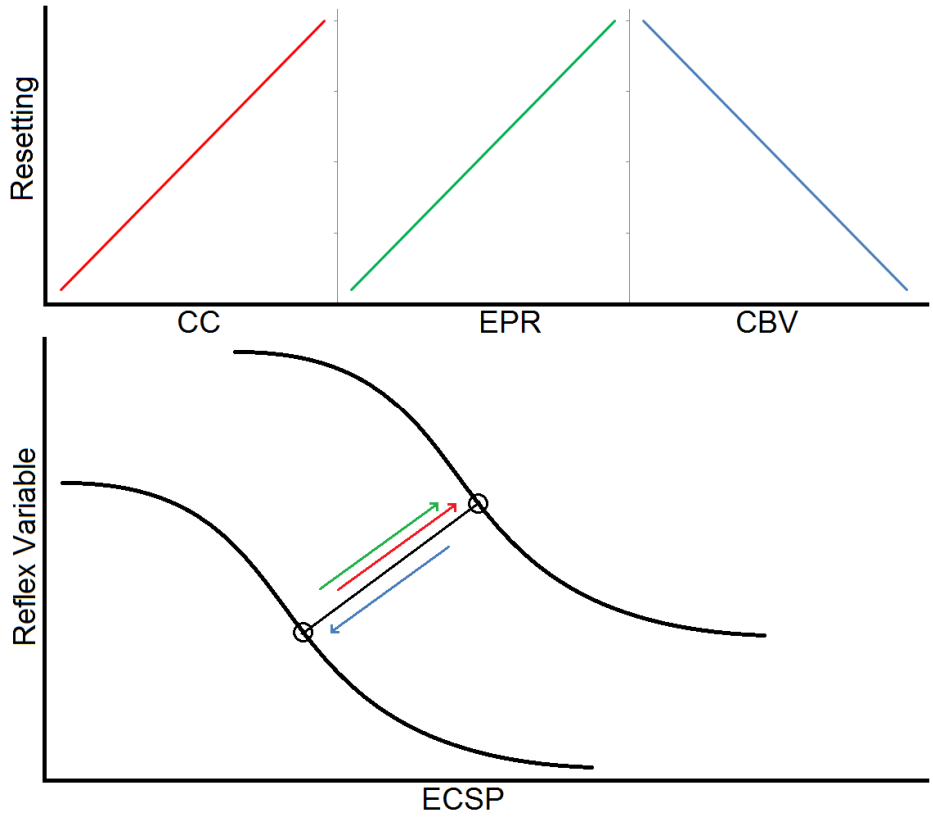
A proposed concept of resetting is in figure 2 in which CC and EPR increase the magnitude of resetting of the ABR, but CBV decreases the magnitude of resetting. Central command input is proportional to the rating of perceived exertion during exercise and EPR is proportional to the amount of work done and the duration of the work. During rest and at the cessation of exercise, CC should become negligible as an input into cardiovascular control, but EPR should remain for a short period of time after exercise. Changes in central blood volume is dependent on orthostasis and muscle pump activity for mean changes in blood volume and on

respiratory pump activity for oscillating changes. Because of the known manipulators of afferent neural control of the cardiovascular system, these aims were devised:

I. **To test the hypothesis** that more upright posture during rest and recovery from exercise increases the ability to modulate cardiovascular function due to cardiopulmonary baroreceptor unloading.

II. **To test the hypothesis** that respiratory influences on blood pressure regulation are attenuated by muscle pump activity during active recovery from prolonged exercise compared to inactive recovery.

Figure 2 Conceptual depiction of the influence of the afferent inputs of the cardiovascular system on resetting of the arterial baroreflex

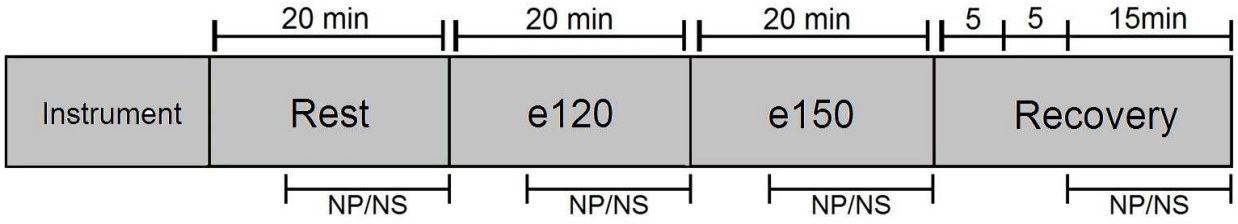


Experimental design

Exercise Testing

Healthy young subjects were recruited to the study. On an orientation day a graded exercise test was performed to measure workload and heart rate correlations so that an estimate of the workload needed to achieve target heart rates on experimental days could be determined. The exercise test also tested the ability of the subjects to maintain endurance and cadence while maintaining a stable upper body position. On the experimental days, subjects were asked to exercise at a cadence of 60 rpm at a workload to maintain a heart rate of 120 bpm (e120) and also 150 bpm (e150) consecutively for 20 minutes each. Then they were allowed to pedal at their own pace with a very low workload for five minutes to cool down after which they rested quietly for five minutes. Then the recovery measurements began.

Figure 3



Neck Pressure/Neck Suction (NP/NS)

Carotid baroreflex function was assessed by our customized NP/NS technique. Heart rate, blood pressure and muscle sympathetic nerve activity baroreflex responses were measured at Rest, e120, e150 and recovery. Acute, 5 second changes in transmural pressure were applied to the carotid sinuses via a chambered neck collar (Potts *et al.*, 1993). The pressures applied

were +40, +20, -20, -40, -60, -80 mmHg repeated 3 times each in randomly assigned order. Heart rate and beat to beat blood pressure were recorded and the stimulus-response graphs were generated for each variable.

Methods

Electrocardiogram (ECG):

On orientation day HR and ECG waveform were measured using a 12-lead ECG to ensure the proper electrical conduction of the cardiac tissue. On experimental day HR and ECG waveform were recorded using a 3-lead ECG in the Lead II configuration. This enabled the measurement of beat-to-beat HR variability and overall analysis of other ECG waveform characteristics of electrical variability.

Neck Pressure/Neck Suction (NP/NS):

Manipulation of the carotid arterial baroreflex function was obtained by a custom built software and hardware configuration. A malleable cushioned neck collar was fitted to the anterior 2/3 of the subject's neck. The collar provides an airtight chamber which encompasses the carotid sinus area. Flexible hoses are attached from the collar to a computer-controlled solenoid that with the help of a vacuum pump can provide positive or negative pressure within the collar chamber. A differential pressure transformer (Validyne, Model MP45-1, Northridge, CA) connected to the collar measures the actual developed chamber pressure (CP) within the collar and relays the signal to the computer display so that the user can adjust power output to maintain the desired CP. When prompted, the computer software initiates positive or negative pressure pulse for 5 seconds. For all proposed studies, pressure will be randomly step-graded between +40 and -80 Torr (20 Torr increments). The positive or negative CP within the collar is

translated into an increase or decrease, respectively, in the extramural tissue pressure which is sensed by the carotid baroreceptors (CB) as a change in carotid sinus vessel wall transmural pressure (Potts *et al.*, 1993). NP is interpreted as hypotension and NS is interpreted as hypertension by the CBs. Beat-to-beat measurements of HR and MAP were analyzed to determine the responses to NP/NS stimulation. Carotid baroreflex (CBR) function curves were drawn using the inverse logistic function as described by Kent et al (Kent *et al.*, 1972). Threshold and saturation calculations were determined by the revised Kent method of McDowall and Dampney (McDowall & Dampney, 2006). On orientation day, all subjects were prescreened with ultrasound and flow-Doppler visualization of the carotid artery and sinus for inappropriate anatomy and arterial blood flow as well as being auscultated for the presence of bruits.

Microneurography:

The path of the radial nerve will be determined using ultrasound imaging. Once the path of the nerve is determined, two small wire electrodes (1 reference, 1 active) will be inserted through the skin. The reference electrode is placed beneath the surface of the skin at an angle of 20 degrees into the skin from the tangent of the skin at the point of entry. The active electrode is inserted into the skin and guided to the nerve bundle by ultrasound imaging. Multiunit MSNA recordings were obtained from the radial nerve of the non-dominant arm approximately 1/3 the length of the upper arm proximal to the elbow. The neural activity was amplified, bandpass filtered (0.7-2kHz), rectified, and resistance/capacitance integrated (time constant 0.1 second) (Iowa Biosystems, Iowa, USA). Neural recordings were accepted 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). After establishing the best possible nerve signal, sympathetic efferent nerve activity was passively recorded.

Statistical Analysis:

One and two way repeated measures ANOVAs will be utilized where appropriate. α will be set at 0.05 such that a $p<0.05$ will be considered significantly significant. When significance is detected with the ANOVA, a student neuman-keuls *post hoc* analysis will be performed to pin point the source of significance. *Post hoc* power analysis will be performed on any statistic determined not to be significant to ensure that a $1-\beta$ is greater than 0.80 so as not to make a type II error.

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CHAPTER II

Autonomic Neural Control of Heart Rate during Dynamic Exercise: Revisited

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Abstract:

The accepted model of autonomic control of heart rate during exercise depicts a withdrawal of parasympathetic activity, which is complete at 100 beats per minute after which the sympathetic nervous system causes subsequent increases in heart rate. However, recent baroreflex modeling data suggests that the parasympathetic nervous system is active and important throughout the spectrum of exercise from rest to maximal workloads. Autonomic control of heart rate in humans was re-evaluated through modeling of new and previously published data involving baroreflex stimulation and pharmacological blockade of the autonomic nervous system. Results show that the parasympathetic nervous system is active throughout exercise and that increases in heart rate from rest through maximal exercise are a result of shifting from a 4:1 vagal-sympatho balance to a 4:1 sympatho-vagal balance. We conclude: i) increases in exercise workload related heart rate are not caused by a total withdrawal of the parasympathetic nervous system followed by an increase in sympathetic tone; ii) accentuated antagonism is key to the shift to sympathetic dominance; and iii) central command induced resetting of the arterial baroreflex causes immediate exercise-onset reflexive increases in heart rate, which are parasympathetically mediated followed by slower increases in sympathetic tone at steady state.

Introduction:

The increases in heart rate (HR) that occur from rest to maximal dynamic exercise rely on a balance between the influence of the parasympathetic and the sympathetic branches of the autonomic nervous system. An early investigation into the autonomic control of HR examined five supine human subjects performing dynamic exercise from rest to maximal oxygen uptake (VO_2max) during: control; complete β-adrenergic receptor blockade of the heart with propranolol; complete parasympathetic blockade of the heart with atropine; and a complete combined autonomic blockade of the heart (i.e. intrinsic HR) (Robinson *et al.*, 1966). From the data obtained the authors concluded that the increases in HR that occurred from rest to mild exercise workloads was primarily a result of a decrease in parasympathetic nervous activity (PSNA), later termed parasympathetic (vagal) withdrawal (Rowell, 1986, 1993). Subsequent workload related increases in HR from mild, or a HR of 100 beats/min exercise workload (a workload at which norepinephrine spillover into the plasma was identified), up to a workload eliciting the individual's HR max was a result of increasing sympathetic nervous activity(SNA) (Rowell, 1986).

Unfortunately, this nuanced interpretation of the mechanisms involved in the exercise induced increases in HR have resulted in a generalized acceptance of the view that the increases in HR that occur from rest to an exercise workload of 100 beats/min result from parasympathetic withdrawal only and that the increases above 100 beats/min are primarily a result of increasing SNA (Rowell, 1993). Unfortunately, an unintended consequence of this interpretation is that it is generally accepted by beginning students of the topic that SNA has very little influence on the HR increases from rest to100 beats/min and that PSNA has no influence on HR increases above 100 beats/min.

However, historical and recent investigations into the autonomic neural control of HR and arterial Blood Pressure (ABP) during dynamic exercise in dogs (O'Leary *et al.*, 1997) and humans (Ogoh *et al.*, 2005) raise into question whether: i) PSNA is withdrawn in the transition from rest to exercise and continues to be withdrawn until it has negligible effect on further HR increases, at approximately 100 beats/min (Matsukawa, 2012); ii) accentuated antagonism can explain the changes in sympatho-vagal balance resulting in the exercise workload related increases in HR (Levy, 1971); and iii) the exercise pressor reflex and the arterial and cardiopulmonary baroreflex control of arterial blood pressure modulation of the exercise workload related changes in sympatho-vagal balance play a role in the operating point pressure of the HR-reflex moving to a point of reduced gain (Fadel & Raven, 2012; Fisher, 2013). Hence, the purpose of this presentation is to revisit and provide a reinterpretation of the well accepted model of the autonomic control of the heart rate that occurs during dynamic exercise from rest to maximum workloads.

Experiments used to obtain the hr modeling data:

We addressed the question of repeatability of the methods used to obtain the data necessary to model the carotid baroreflex function curves by recruiting 9 healthy human subjects in 2012 to repeat the exact same control resting and exercise protocols in Fort Worth that were performed in Copenhagen in 2004 by a different 8 human subjects, the data of which were published in 2005 (Ogoh *et al.*, 2005). There was no statistically significant difference in HR between the Copenhagen data and the Ft Worth data when the means were compared between the studies at each workload of exercise, therefore the control data were considered as coming from one population.

Hence, the data utilized in the current presentation for modeling the HR response from rest to progressive increases in dynamic exercise workloads in the control condition was obtained from the 17 subjects (n= 14 men and 3 women) with a mean age of 25 ± 2.5 years, height 181 ± 7cm, and weight 80 ± 10 Kg (mean ± SD). However, only the data from 8 (6 men and 2 women) with a mean age of 25 +/- 2years, height 181+/- 7cm and weight of 71+/- 3 Kg were involved in the autonomic blockade studies performed in Copenhagen.

All subjects were free of any known cardiovascular and pulmonary disorders, and were not using prescribed or over-the-counter medications. Each subject provided written informed consent as approved by The Ethics Committee of Copenhagen (KF01-369/97) or the Institutional Review Board at the University of North Texas Health Science Center (IRB #2010-058). All experiments were performed in accordance with the Declaration of Helsinki. In both institutions each of the subjects were requested to abstain from caffeinated beverages for 12 h, and strenuous physical activity and alcohol for at least a day. Subjects performed a progressively graded increase in workload exercise test on a cycle ergometer to determine workloads at HRs of 90, 120 and 150 beats/min to be used during the experimental treatments.

On experimental days and after 30 min of resting data collection, each subject performed 25 min bouts of exercise at steady-state HRs of 90 (EX90), 120 (EX120), and 150 (EX150) beats/min. The beat-to-beat HR and MAP responses to the NP/NS protocol during steady state rest, EX90, EX120, and EX 150 during the control, complete metoprolol (β_1-adrenergic receptor) blockade and complete glycopyrrolate (peripheral muscarinic receptor) blockade experiments were recorded, details of the autonomic blockade were previously reported by Ogoh et al. (Ogoh *et al.*, 2005). The data were used to assess the carotid-HR baroreflex at each stage of the protocol.

Construction of the model of hr increases from rest to maximal exercise:

a) Control (Sympatho-Vagal balanced) HR response to exercise and NP/NS:

The specifics of the data analyses and construction of the carotid-HR baroreflex functions curves were previously published (Ogoh *et al.*, 2005). In the present review, the data were analyzed as described below. The HR at the operating point (OP) pressures at rest, EX90, EX120 and EX150 without autonomic blockade were plotted against their related exercise workloads and fit to a linear regression line that included an extrapolated HR at maximal workload (r^2=0.99).This HR/workload regression line is representative of the changes in the sympatho-vagal balance without autonomic blockade (Figure 1A, solid red line).

The HRs at carotid-HR baroreflex threshold and saturation pressures were plotted against rest, EX90, EX120 and EX150 workloads. A linear regression relationship between HRs and related workloads existed for the threshold point pressures (solid green line, r^2=0.97). A non-linear best-fit regression relationship existed between the HRs and related workloads at the saturation point pressures (solid blue line). This required the use of non-linear modeling analyses to identify that the line of best fit was a 3rd order polynomial regression (r^2=0.96, figure 1A).

b.) Autonomic neural influences on HR response to exercise and NP/NS:

i.) Control Condition (without autonomic blockade)

The differences between the operating point regression line, i.e. the sympatho-vagal balance line, and the saturation point regression line, maximum mechanically stimulated decrease in HR, is the NS induced effective parasympathetic reserve at any given workload (figure 1A). The difference between the operating point regression line and the threshold point regression line, maximum mechanically stimulated increase in HR by NP, is effective parasympathetic

tone at any given workload (figure 1A). The difference between the saturation line and threshold line represents the overall NP/NS induced functional reflexive influence of the parasympathetic nervous system (PSNS) on HR (figure 1A).

ii.) Cardiac muscarinic receptor blockade condition

The HRs recorded during parasympathetic cardiac blockade by glycopyrrolate were plotted against their related workloads (dashed green line) and exhibited a linear relationship (r^2=0.99, figure 1B).The difference between the operating point regression line (solid red line) and the max vagal inhibition line represents the PSNS contribution to the steady state HR from rest to maximal exercise.

iii.) Cardiac β_1-adrenergic receptor blockade condition

The HRs during maximal cardiac β_1-adrenergic receptor blockade with metoprolol were plotted against their related workloads and exhibited a linear regression line of best fit (dashed blue line, r^2=0.99, figure 1B). The difference between the sympatho-vagal balance line and the max sympathetic inhibition line represents the SNS contribution to the steady state HR from rest to maximal exercise (figure 1B).

c) Summary of modeling outcomes

i.) The steady state HR gets closer to the unopposed sympathetic HR as the workload increases indicating a stronger influence of the SNS at higher workloads but because it never reaches the same value there is never a complete absence of parasympathetic influence (figure 1B).

ii.) The relative influence of each branch of the autonomic nervous system starts with a parasympathetic dominant, 4:1 ratio PSNS to SNS, influence at rest and as exercise workload

increases there is a shift to a more sympathetic, 1:4 PSNS to SNS, influence at 175 bpm (figure 2).

iii.) According to the selective PSNS and SNS blockade studies, the PSNS and SNS efferent activity at the sino-atrial node reach an equal bpm influence on HR at approximately 140bpm. There is a linear increase in SNS influence from 8.9 bpm to 14 bpm from rest to maximum exercise and a linear decrease in PSNS influence from 34.7 bpm to 3.4 bpm, rest to maximum exercise. However, the change in the PSNS:SNS ratio is mainly due to a greater linear decrease in the parasympathetic influence on steady state HR at the exercising workloads.

Discussion:

Between the years of 1886 and 2012, a large number of animal and human investigations established the presence of three integrated autonomic neural mechanisms, Central Command (CC), the Exercise Pressor Reflex (EPR) and the Arterial Baroreflex (ABR) to be involved in arterial blood pressure regulation during dynamic exercise. The specific investigations are documented in a number of comprehensive reviews that identify that an exercise workload related activation of CC and EPR from both central and peripheral neural inputs result in appropriate workload related efferent cardiorespiratory responses (Shepherd, 1981; Mitchell, 1990; Rowell, 1993; Raven *et al.*, 2006). During progressive increases in exercise workloads from exercise onset to maximal exercise, the hemodynamic responses are directly related to the workloads and the individual's rating of perception of effort (RPE) (Williamson *et al.*, 2001). For many years a simple concept of PSNS (vagal) withdrawal was proposed to be the mechanism by which HR increases at exercise onset and when the exercise workloads were progressively increased up to HRs of 100 bpm (Rowell, 1986, 1993). Subsequent increases in workloads resulted in HR increases >100 bpm and were directly related to increases in SNA.

In the present review, we add more relevant published findings and a reanalysis of our published data (Ogoh *et al.,* 2005) in order to re-examine Rowell's (Rowell, 1993) simplified representation of Robinson et al.'s (Robinson *et al.,* 1966) initial study in which selective pharmacologic blockade and double blockade of the parasympathetic (PS) and sympathetic (S) arms of the autonomic control of HR of the exercising human was accomplished. The new information resulting from our analyses indicates that, contrary to the model suggested by Rowell (Rowell, 1993), a balance between the S and PS neural control of the HR responses to progressive increase in exercise workloads remains viable and effective from rest to maximum, see figure 3. In addition, the data from the blockade studies indicate that the PSNS influence on HR is necessary for reflex control, whereas, the SNS is required to set the workload related steady state HR. Furthermore, our additional data analyses identify a more refined model of the progressive exercise workload/HR related changes in sympatho-vagal balance.

Therefore, in contrast to the simple vagal withdrawal concept at exercise onset, we propose that the HR increases at the onset of exercise and with the increases in exercise workloads, are related to an ABP regulatory mechanism (Rowell, 1986, 1993). The proposed ABP regulatory mechanism requires CC to actively reset the "Set Point" of the ABR to allow the ABP to rise at the same time that HR is increasing to increase cardiac output and raise ABP (Williamson *et al.,* 2001). During progressive increases in dynamic exercise workloads, ABR resetting is linearly related to the increase in the exercise workloads (Fadel & Raven, 2012). At exercise onset the immediate act of resetting of the "Set Point" reference pressure of the ABR results in negative feedback from the arterial baroreceptors to the reference ABR 'set point' within the nucleus tractus solitarius (NTS) indicating that the OP pressure of the reflex is too low and initiates the rapid responding inter-beat withdrawal of the PS modulation of the HR at the

sino-atrial node. At the same time the slower responding increase (sec.) in central SNA outflow progressively inhibits PS tone and modulation at the S-A node (Ogoh *et al.,* 2005) by accentuated antagonism (Levy, 1971; Uijtdehaage & Thayer, 2000), and the increased SNA becomes the major drive to increase HR (figure 4). As the exercise workloads progressively increase, the increases in SNA (Hartley *et al.*, 1972; Savard *et al.*, 1989) progressively inhibit PS modulation of the HR at S-A node up to maximum workloads. The exercise workloads related increase in SNA is related to the increases in CC (McIlveen *et al.,* 2001; Fadel & Raven, 2012) and the EPR (McIlveen *et al.*, 2001; Fisher, 2013). However, the workload related increases in SNA are modulated by the increased central blood volume's (CBV) loading of the CPBRs afferent inhibition of central sympathetic outflow (Fadel & Raven, 2012).

We propose that the mechanism by which the required continuous increase in workload related HRs is accentuated antagonism (AA), sometimes called 'cooperative antagonism'. For the Autonomic Nervous System (ANS) this mechanism is where one branch of the ANS works to oppose the effect of the other branch of the ANS in the central nervous system (CNS) and at the end organ in the peripheral nervous system (PNS). Ostensibly the inhibition in the periphery is required so as to not have competitive effects taking place within an organ with dual innervations (Levy, 1971). At the S-A node the AA mechanism requires that a portion of the norepinephrine released at the sympathetic nerve terminal binds to the β adrenoreceptors on the post-ganglionic parasympathetic neurons inhibiting the release of acetylcholine (Ach), thereby, accentuating the increase in HR for a given amount of SNA. Conversely Ach binds muscarinic receptors on the sympathetic post-ganglionic neurons to inhibit the release of norepinephrine (Uijtdehaage & Thayer, 2000) and accentuating the decrease in HR for a given amount of PSNA.

This mechanism is useful in reflexive actions, such as the arterial baroreflex, where changes in (HR) and cardiac output (Q) are adjusted on a beat to beat basis (Levy, 1971).

In the transition from rest to progressive increases in exercise workloads, our analysis identifies that the dynamic influence on HR of the sympatho-vagal balance progresses to a sympatho-dominant balance but not before an initial increase in a PS dominance resulting from CPBR inhibition of SNA (figure 3). The presence of this SNA inhibition following the onset of exercise was confirmed, when it was reported that MSNA briefly decreased at the onset of leg exercise returning to and then exceeding baseline at approximately 140 beats per minute (Ichinose *et al.*, 2008). In addition, the CBR-HR response range is increased at the lower exercise workloads (figure 1A) supporting the argument that accentuated antagonism is a dominant mechanism in the sympatho-vagal control of HR throughout progressive increases in exercise workloads.

Sympatho-vagal balance has traditionally been thought of as akin to a see-saw on a playground. When one side goes up the other comes down. During exercise the fulcrum of that see-saw is elevated (resetting) so that the blood pressures eliciting the baroreflexes are allowed to be higher for increased steady state conditions and the same reflexive magnitude changes, see Fig 3. This is evident when studying the data of Potts (Potts *et al.*, 1993), Fadel (Fadel *et al.*, 2001), Volianitis (Volianitis *et al.*, 2004), and Ogoh (Ogoh *et al.*, 2005) all of whom show the ABR resetting to higher blood pressures as exercise workloads increase. The mechanism for the resetting of the baroreflexes has not been completely identified. CC has a significant role in the fulcrum of the see-saw being reset to a higher OP pressure, resulting in an increase in sympathetic dominance on the system and a subsequent decrease in the ability of the PSNS to modulate HR (figure 3). As reported by Ogoh (Ogoh *et al.*, 2005), baroreflex changes in beat to

beat HR are due to the PSNS and this shift to a more sympathetically dominant interaction at higher workloads of exercise would explain the decrease in response range of the carotid-HR baroreflex.

More recently other neuromodulators, such as nitric oxide, neuropeptide Y and natriuretic peptides within the heart act as co-transmitters and interact in the neuronal cyclic nucleotide-dependent pathways to modulate PSNA and SNA locally (Mohan *et al.*, 2000). It has been reported that SNA mediated HR changes are dependent on a pre-ganglionic mechanism involving nitric oxide (NO) (Mohan *et al.*, 2000).This mechanism was found to be enhanced after exercise training causing a reduction in the cardiac sympathetic response. Albeit, it was found that the NO influence on chronotropic response after exercise training was probably minimal because there was still a significant decrease in training response even with NO inhibition (Mohan *et al.*, 2000). Whether or not local production of reactive oxygen species (ROS) at the S-A node scavenges the local NO allowing increased SNA resulting in a greater antagonism of the PSNS remains obscure. Another recently identified set of neuromodulators of the SNA and PSNA with the mechanistic potential to inhibit PSNA is the change in opioid receptor populations and locally produced enkaphalins on the S-A node (Napier *et al.*, 1998; Farias *et al.*, 2001; Jackson *et al.*, 2001). However, what role the opioid neuromodulators play in further accentuating the SNA/PSNA antagonism has yet to be determined.

Additional support of there being an active sympatho-vagal balance at exercise onset were reported by Takahashi et al. (Takahashi *et al.*, 2004) in which that the response in HR during voluntary static arm exercise in tetraplegics, who lack supraspinal sympathoadrenal control but have intact vagal control, do not have the same increases in HR associated with the static exercise in healthy subjects. Therefore, if vagal withdrawal were the primary mechanism

by which HR increased at exercise onset, the increase in HR at the onset of static exercise in the tetraplegic subjects with intact PSNS would be the same as in normal subjects. The fact that the HR response of the tetraplegics was less than the healthy normal subjects identified that increases in SNA and the presence of a sympatho-vagal balance was required for the physiological HR response of healthy subjects to occur (Takahashi *et al.*, 2004). .

However, Kadowaki et al. (Kadowaki *et al.*, 2011; Matsukawa, 2012) recently demonstrated using the fictive exercising decerebrate cat model that both cardiac sympathetic and parasympathetic neuron activity were increased at the onset of spontaneous fictive movement. One explanation for this paradoxical increase in both branches of the ANS at exercise onset is that it is a redundant mechanism to protect against an exacerbated increase in HR due to immediate CC induced increases in cardiac-SNA. Unfortunately, without measurements of neurotransmitter release at the end organ, the assumption of the presence of redundant mechanisms could not be confirmed. Furthermore, it has been reported that MSNA decreases at the onset of dynamic leg cycling (Saito *et al.*, 1993; Ichinose *et al.*, 2008) as a result of the muscle pump increasing venous return and loading the CPBRs (Saito *et al.*, 1993). Hence, it is possible that Kadowaki et al.'s findings (Kadowaki *et al.*, 2011; Matsukawa, 2012) indicate the presence of a differential control of muscle- and cardiac-SNA, or, in the non-physiological decerebrate cat studies resulting in fictive movements, the muscle pump activity may not be of sufficient strength to increase venous return to load the CPBRs similarly as to that observed in the exercising humans. The lack of CPBR loading would account for the increases in cardiac-SNA at the start of fictive movement seen in the cats (Matsukawa, 2012) and which could be attributed to CC increase in SNA without the feedback inhibition resulting from the loaded CPBR. However, the increase in PSNA at the onset of fictive movement identified by

Kadowakie et al. (Kadowaki *et al.*, 2011; Matsukawa, 2012) is contrary to the concept of CC related ABR resetting to an increased 'set point' pressure and the vagal withdrawal mediated increase in HR.

Another confounding issue identifying whether the PS influence on exercising HR is withdrawn or inhibited is the large number of published heart rate variability (HRV) studies concluding that exercise induced reduction in power spectral high frequency (HF) peaks indicates PS withdrawal with the increasing exercise workloads. Because in human studies there is no safe accessible PS neuron for nerve recordings, the measure of HRV is often used as an indicator of PSNA. It is well known that in healthy and diseased subjects, increased PS control of the heart at rest can be detected by time and frequency domain indices of HRV, especially in the high frequency (HF) peaks (1996). However, a common error of interpretation is that the HF peaks in HRV spectra are indicators of "PS tone", when in fact a more accurate and acceptable description is that it describes "PS modulation"(1996). For example, drugs that inhibit cardiac muscarinic receptors on the SA node (atropine or glycopyrrolate) cause the HF peak to disappear as if due to a "withdrawal of PS tone". Only when the opposite is done through pharmacological or electrical stimulation of the vagus nerve to increase the PS tone and the HF peak also disappears, we realize that changes in the HF peaks do not indicate changes in PS tone (Napier *et al.*, 1998). Therefore, what is actually identified by the change in the HF peak is not PS tone, but PS modulation. Furthermore, during exercise, increases in breathing frequency shifts the HF peak to the frequency of breathing, thereby potentially eliminating the expected HF peak and leading to false conclusions about decreased PS tone. Hence, we question whether the withdrawal of PSNA from rest to progressive increases in exercise workloads is the main mechanism explaining exercise related increases in HR. Current perspectives and animal data

suggest that this is not the case. In contrast, we suggest that there is a decrease in the PSNS's modulation effect on HR during exercise due to the increased SNA inhibiting the PSNA at the S-A node.

Conclusion:

Our proposed model (figure 4) depicts the role of the parasympathetic and sympathetic nervous systems throughout all workloads of exercise. The research points to a system of control that does not have any clear on/off thresholds, but a continuum of balanced control (Figure 3). It is important to think of sympatho-vagal balance as not being the equal influence of two branches of the autonomic nervous system but the interplay between the two for short term and long term modulation of HR while keeping in mind that the PSNS is quick and short lived, but the SNS is stronger and has the ability to attenuate the PSNS influence. As exercise is initiated, CC resets the ABR which is immediately met by decreased PSNA and a slight decrease in SNA due to increased venous return in phase 1 (Wasserman, 1994) and loading of the CPBRs, which enables the PSNS to exert more modulation on the heart. As exercise workload increases, the parallel increases in CC and EPR increases the ABR resetting which in turn augments the SNA increases in HR and depresses the PSNA HR reflex response.

Further research will be required to elucidate the nervous system outputs versus functional contribution to changes in heart rate during exercise. A particular confounding factor will be the multiple linkages within the intrathoracic ganglia which exert an effect on cardiodynamics that render the sum greater than the individual parts, making *in situ* examination essential (Armour, 2004). *In situ* examination of parasympathetic nervous activity in humans will remain difficult until better methods of direct nerve recordings are developed.

Perspective:

It is well established that age related reductions in maximal HR and β-1adrenergic receptor blockade resulting in reduced maximal and sub-maximal HRs for cardiac patients are symptoms of chronotropic incompetence (CI). In addition, we noted in our discussion that tetraplegics, who lack supraspinal sympathoadrenal control but have intact vagal control, also exhibit CI. More recently Mendonca et al. (Mendonca *et al.,* 2011) identified that individuals with Down syndrome exhibit CI and autonomic dysfunction. Our re-analysis of recently published data (Ogoh *et al.*, 2005) in conjunction with a review of the previous published data, identifies that CI may be related to a functional inhibition of the PSNA by the SNA mechanisms of accentuated antagonism, cellular mechanisms involving NO, neuropeptide Y and natriuretic peptides affecting neuronal cyclic nucleotide-dependent pathways and opiod receptors in autonomic neurons and the S-A node (Mohan *et al.*, 2000; Uijtdehaage & Thayer, 2000; Farias *et al.*, 2001; Jackson *et al.*, 2001; Adlam *et al.*, 2012).

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Figure legends

Figure 1

A) Modeling of the reflexive control of heart rate by the carotid baroreflex. Steady state HR at each workload represents the sympatho-vagal balance (solid red line). Increases and decreases in HR by simulated hypo- and hypertension induced by neck pressure/suction (green and blue lines, respectively) are plotted against workload. These data indicate that the parasympathetic influence on the reflex control of HR is diminished as the exercise workloads increase from rest to max exercise.

B) Modeling of pharmacological blockade studies of each branch of the autonomic nervous system from rest to maximal exercise. Steady state HR at each workload without blockade represents sympatho-vagal balance (solid red line). Steady state HR at each workload with muscarinic blockade by glycopyrrolate (green dashed line) and β_1-adrenergic blockade by metoprolol (blue dashed line) represent the un-opposed sympathetic HR and un-opposed parasympathetic HR, respectively. As HR increases with workload, the sympatho-vagal balance approaches a more sympathetically dominant balance.

Figure 2 Depicted are the % contributions to HR by each branch of the autonomic nervous system determined by autonomic blockade studies. The PSNS (Dashed Line) contributes 80% influence to the resting HR and the SNS (Solid Line) contributes the other 20%. There is an equal contribution to both branches at near 140 bpm

after which the ratio changes quickly to a more sympathetically dominant system. The respective lines indicate the change in HR from a single branch's selective autonomic blockade as a percent change in HR of the sum of the absolute values of HR change of both branches.

Figure 3 At rest as well as throughout exercise, there is a sympatho-vagal balance. The parasympathetic functional control is related to the sympathetic tone while maintaining a balance. Relative size of "SNA" on the right side of the see saw affects the reflexive control of the PSNA on the left side as depicted by size of the arrows. As exercise is initiated, there is a slight decrease in sympathetic tone due to the loading of the CPBR which allows for the parasympathetic nervous system to exert more control modulation on the heart. Along with this initial decrease in sympathetic tone is a transient decrease in BP so the HR is increased reflexively by lessening of parasympathetic tone to maintain and increase cardiac output. As exercise workload increases, central command and exercise pressor reflexes force increases in sympathetic tone causing a further rise in HR and a depression of parasympathetically mediated HR reflex response.

Figure 4 Modified version of the diagram proposed by Rowell (figure 5-4 (Rowell, 1993)) depicting a continuum of autonomic influence from both branches of the autonomic nervous system throughout exercise. The area with the diagonal lines represents the sympathetic influence at all workloads of exercise. The dotted area represents the functional parasympathetic modulation of HR at all workloads of exercise. The center line is the relative HR, i.e. dynamic sympatho-vagal balance.

Figure 1

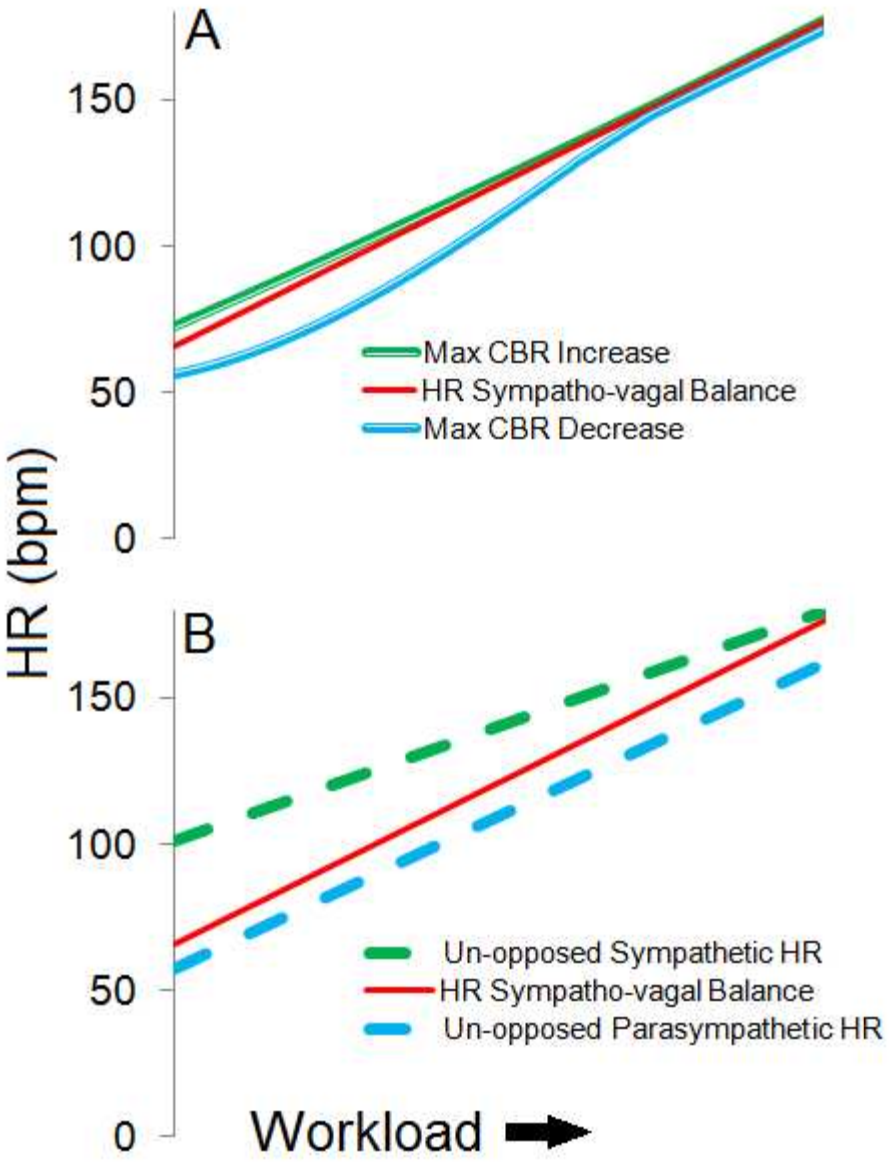

A
150
100
50
0
Max CBR Increase
HR Sympatho-vagal Balance
Max CBR Decrease
HR (bpm)
B
150
100
50
0
Un-opposed Sympathetic HR
HR Sympatho-vagal Balance
Un-opposed Parasympathetic HR
Workload

Figure 2

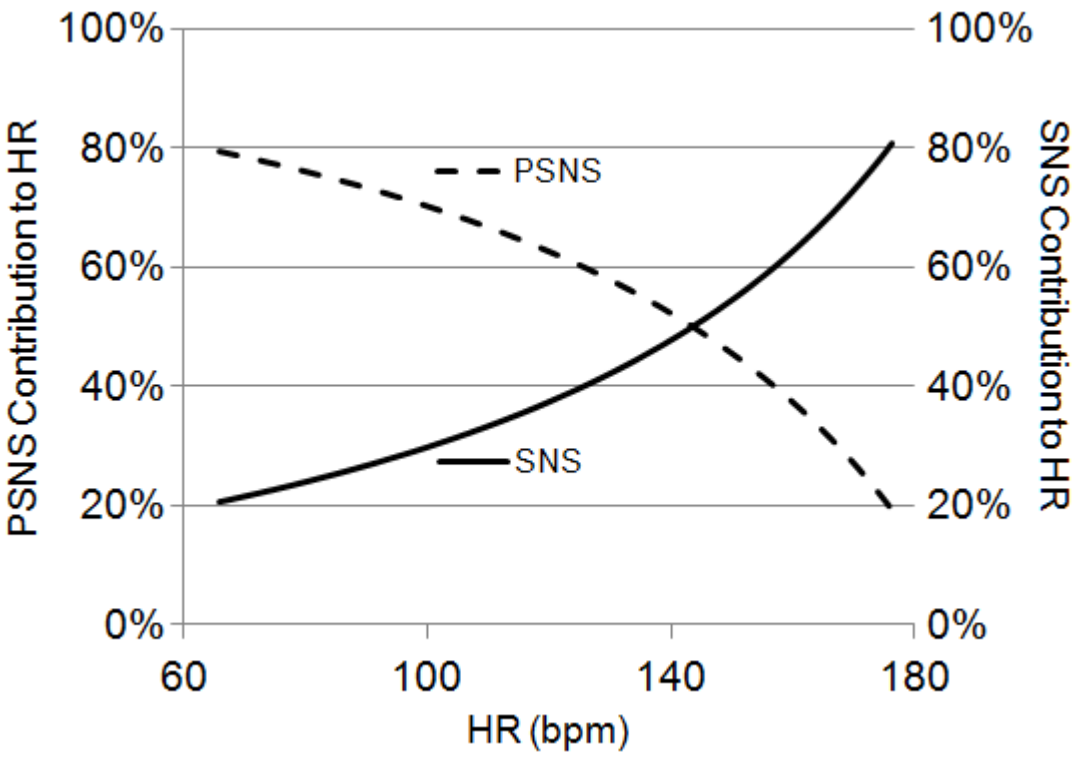

100%
80%
60%
40%
20%
0%
PSNS Contribution to HR
PSNS
SNS
SNS Contribution to HR
60
100
140
180
HR (bpm)

Figure 3

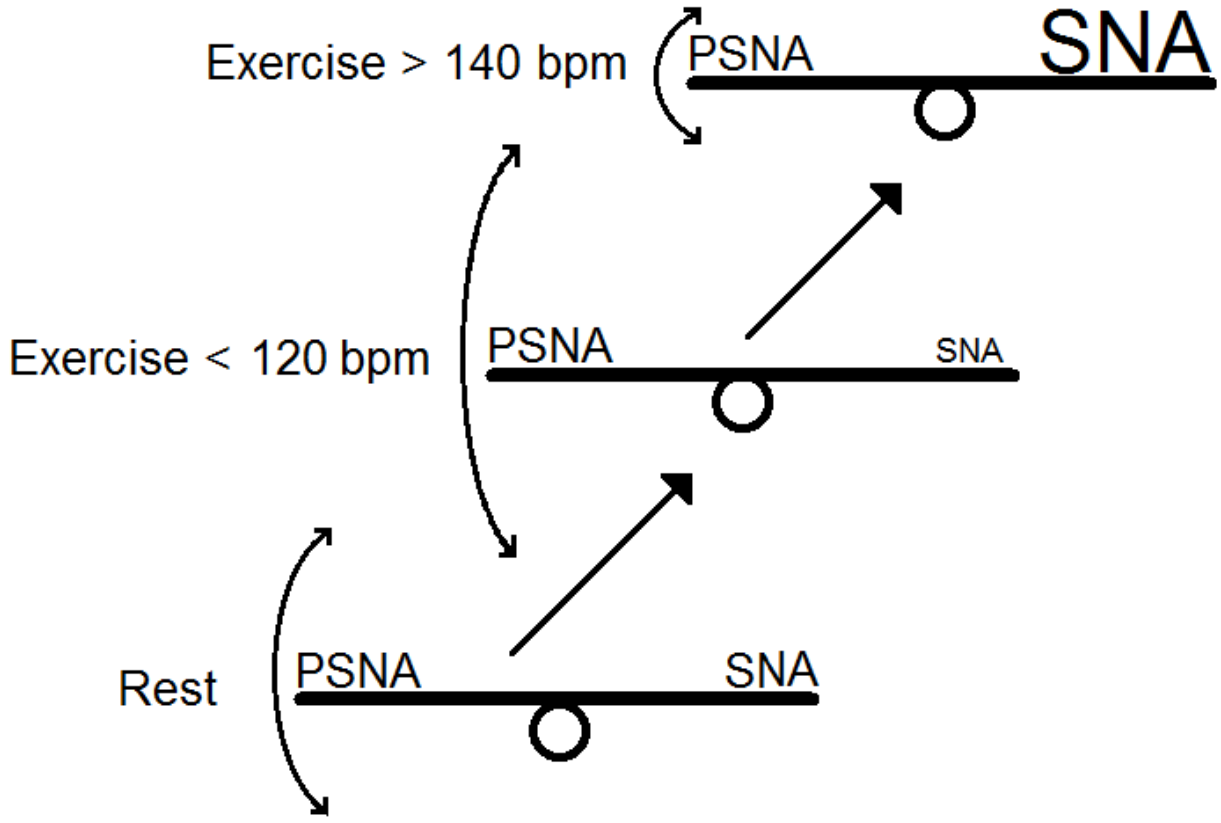
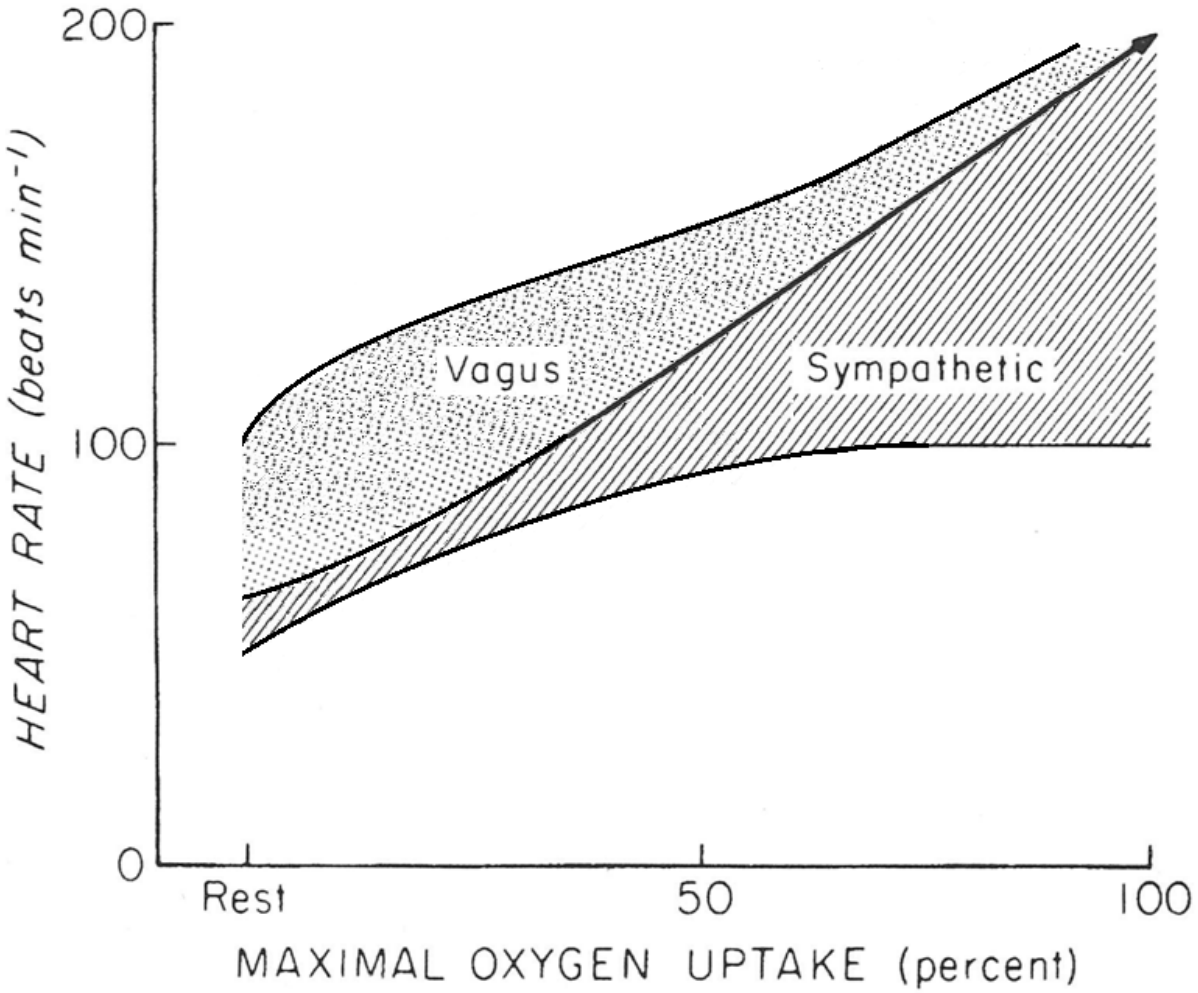

Exercise > 140 bpm
PSNA
SNA
Exercise < 120 bpm
PSNA
SNA
Rest
PSNA
SNA

Figure 4


200
100
0
HEART RATE (beats min⁻¹)
Vagus
Sympathetic
Rest
50
100
MAXIMAL OXYGEN UPTAKE (percent)

CHAPTER III

Autonomic neural control of heart rate prior to and immediately following cycling exercise: upright versus semi-recumbent posture

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Journal of Physiology

(submitted)

Abstract:

Immediately upon cessation of exercise, heart rate (HR) and arterial pressure (AP) start to decrease toward resting values. Inappropriate decreases in both HR and AP lead to syncope. Cardiovascular control in the moments following exercise has been explored extensively; however, in humans there remains a gap in the literature regarding direct neural recordings early after prolonged large muscle group dynamic exercise. We tested the hypothesis that baroreflex control of HR in early recovery from moderate to heavy exercise is dependent on posture loading of the cardiopulmonary baroreceptors. Furthermore, we investigated the effects of post exercise unloading of the cardiopulmonary baroreceptors on muscle sympathetic nerve activity. We found that position significantly affects the characteristics of the carotid baroreflex control of HR. Also, MSNA is significantly increased during recovery in the semi-recumbent position compared to pre-exercise rest. This is the first study to continuously record MSNA during rest, through heavy two-legged cycling exercise and during recovery. We conclude that postural loading of the cardiopulmonary baroreceptors influences cardiovascular control before and immediately after heavy dynamic leg cycling exercise and that muscle sympathetic nerve activity is increased during early recovery with post exercise hypotension.

Introduction:

Since the early 1900's numerous questions have surrounded the neural regulatory mechanisms involved in the heart rate (HR) increases that occur at the onset of exercise and during the progressive increases in dynamic exercise workloads up to maximum intensity (Rowell, 1993). In the past 20 years it has been established that the operating point (OP) pressure of arterial baroreflex regulation of mean arterial blood pressure (MAP) is reset upwards and rightwards in direct linear relation to the increasing workloads (Raven *et al.*, 2006). Recent evidence identifies that the increases in HR required by the resetting of the OP pressure are the result of the progressive increase in sympathetic dominance of the sympatho-vagal balance which is the primary mechanism producing the workload proportional increase in HR (Ogoh *et al.*, 2005). Prior to the 1950s, recovery HRs following many types of standardized exercise stress tests were used to provide assessments of cardiovascular fitness. Early examples of using recovery HRs for estimating an individual's cardiovascular fitness was the Harvard Step Test (Gallagher & Brouha, 1943) and another for estimating clinical risk of heart disease was the Master's two-step (Master, 1950). Subsequently, with the introduction of exercise stress testing that utilized bicycle ergometers and motor driven treadmills and continuous electrocardiogram (ECG) recordings during the testing, the fitness predictions and cardiovascular risk estimates from recovery HRs have fallen into abeyance. The neural regulatory mechanisms involved at the onset of exercise and during HR recovery have recently been reviewed by Coote (Coote, 2010). However, this review was focused on identifying the mechanisms underlying exercise induced increases in HR and on the intermittent recovery HRs of Olympic Biathletes during competition.

Beginning in 1990 Raven's group sought to answer the question, "why does the arterial baroreflex allow both HR and MAP to increase at exercise onset and throughout a progressive

increase in workload exercise stress test?" The initial experiments using human subjects performing dynamic exercise investigated the model of arterial baroreflex resetting proposed by Rowell and O'Leary (Rowell & O'Leary, 1990), which was based on earlier work using animals and humans (Bevegard & Shepherd, 1966; Rowell, 1974; Melcher & Donald, 1981; Walgenbach *et al.*, 1981; Walgenbach & Donald, 1983a, b; Sheriff *et al.*, 1990) to suggest that arterial baroreflexes were reset. A historical account of the investigations performed by many groups to examine the underlying question as to whether "arterial baroreflexes are reset or switched off" is summarized in a recent review (Fadel & Raven, 2012). In summary, exercise workload related arterial baroreflex resetting is a consequence of the interaction between central command's feed-forward and the exercise pressor reflex's feedback mechanisms that are continuously modulated by the arterial and cardiopulmonary baroreflexes. The bulk of the evidence confirms the primary finding of Sherriff *et al*.'s earlier work (Sheriff *et al.*, 1990) that the teleological rationale for resetting of the arterial baroreflex during exercise is to provide an exercise intensity-related brake on the hypertensive drive emanating from the exercise pressor reflex, while at the same time allowing adequate perfusion to the exercising muscles.

Our understanding of the neural mechanisms involved in downward resetting of the arterial baroreflex during reduced workload, cessation of work and recovery from exercise is, however, still incomplete. One investigation into the changes in HR and MAP that occur during recovery identified that the mode of recovery (inactive, active or passive) plays a significant role in the maintenance of cardiac output and mean arterial pressure following short duration (3min) upright submaximal cycling exercise (Carter *et al.*, 1999). Furthermore, the authors indicated that their data suggests that central command does not appear to play an important role in maintaining blood pressure and cardiac output during recovery, but probably does influence the

recovery heart rate (Carter *et al.*, 1999). Halliwill *et al*. (Halliwill *et al.*, 2013) summarized his group's work since 2001 in which they investigated potential mechanisms underlying post-exercise hypotension during 60 mins of supine recovery after performing a standard, upright, 60% peak oxygen uptake (VO_2peak) exercise. Their cumulative findings identified that skeletal muscle afferents are involved in the arterial baroreflex post-exercise downward resetting by affecting discrete receptor changes within the nucleus tractus solitarius (NTS). Simultaneously, post-exercise skeletal muscle vasodilatation is a result of histamine H_1 and H_2 receptor activation (Halliwill *et al.*, 2013). Supine posture-related increases in central blood volume (CBV) decrease muscle sympathetic nerve activity (MSNA), whereas decreases in CBV due to upright posture increase the OP pressure of the Carotid Baroreflex (CBR) (Pawelczyk & Raven, 1989; Ogoh *et al.*, 2003; Ogoh *et al.*, 2005). Hence, as most human activities of daily living and athletic performance rarely recover in the supine position, further investigation into recovery posture is required.

Ogoh recently demonstrated that beat to beat reflexive changes in HR at all intensities of exercise are entirely parasympathetic in nature (Ogoh *et al.*, 2005), however, there are conflicting reports on parasympathetic nervous system function during early recovery from prolonged exercise in humans (Savin *et al.*, 1982; Kannankeril & Goldberger, 2002; Kannankeril *et al.*, 2004; Pierpont & Voth, 2004; Coote, 2010). A reduction in MSNA at 60 minutes post-exercise in borderline hypertensive men has been identified (Floras *et al.*, 1989), while Halliwill *et al*. (Halliwill *et al.*, 1996) has reported an attenuation of the transduction of MSNA to the blood vessel wall at 60 minutes into supine recovery. However, there have been no direct neural recordings demonstrating the characteristics of MSNA in healthy humans during early recovery from prolonged leg cycling exercise. It is well established that there exists accentuated

antagonistic interactions of the parasympathetic and sympathetic branches of the ANS in controlling HR (Levy, 1971). Accordingly, we hypothesize that the greater unloading of the cardiopulmonary baroreceptors would accentuate arterial baroreflex control of HR and MSNA response during upright recovery compared to rest. To test this hypothesis we compared carotid baroreflex responses during rest and recovery from exercise in both the upright and semi-recumbent positions.

Methods:

9 healthy human subjects (1 female) aged 24.4 years (±1.16) volunteered to participate in protocol 1 of the study. Another 13 healthy human subjects (3 female) aged 25.5 years (±0.88) volunteered for protocol 2 of the study. On an orientation/familiarization day, each subject completed the Institutional Review Board (IRB) approved consent paperwork and a health history questionnaire (IRB#2010-058). On the same day each subject underwent a physical examination and graded exercise test. The physical examination included 12-lead ECG and automated blood pressure auscultation (Suntech Tango+, Morrisville, NC) in both the sitting and upright standing positions. Anatomical location of and flow through the carotid sinuses was inspected by ultrasound and the locations were auscultated for bruits. Upon completion of the physical examination the subjects for protocol 1 of the study mounted an upright cycle ergometer (Scifit ID5500, Tulsa, OK) and were instrumented for 3-lead ECG and beat to beat blood pressure measurement with the Finometer (Finapres Medical Systems, Amsterdam). After ECG and blood pressure signals were acquired and calibrated the subject was fit with a malleable open chambered neck collar which enclosed the anterior 2/3 of the neck from mandible to clavicle. A familiarization session with the neck pressure/neck suction protocol followed in which the subject practiced end-respiratory breath holds and free breathing while being stimulated with

either pressure or suction. At least one stimulation of each pressure or suction intensity (-80, -60, -40, -20, +20, +40 mmHg) was applied to each subject. Following the NP/NS familiarization the subject performed a graded exercise stress test. Each subject started at a very light workload and was asked to maintain a pedaling cadence of 60 rpm as the workload was step increased every 2 minutes. The graded exercise stress test was concluded when the subject was unable to continue despite strong verbal encouragement or was no longer able to maintain 60 rpm. Each subjects HR at each workload was recorded and an individual HR/workload regression line was used to determine the subject's workloads necessary to elicit each subject's heart rate of 120 (e120) and 150 (e150) beats per minute. Subjects for part 2 of the study underwent the same NP/NS familiarization and graded exercise stress test protocol in 70˚ from horizontal back supported semi-recumbent position.

No less than 2 days later the subjects returned for the experimental exercise protocol. For protocol 1, subjects were asked to sit upright on the cycle ergometer at rest. Their ECG, HR and blood pressure were recorded. Subjects were fitted with the neck collar and then asked to relax. NP/NS protocol was performed according to Potts *et al* (Potts *et al.*, 1993) during end respiratory breath holds at rest, and during free breathing at steady state exercising HRs of 120 and 150 bpm, and during inactive recovery from exercise. Total time of exercise was 56.87 minutes (±1.31 min, SEM).

For protocol 2, subjects were asked to sit in a 70˚ back supported semi-recumbent position with legs extended horizontally on a cycle ergometer (Intellifit Psycle, Bedford, Texas) and instrumented for beat to beat ECG, HR and blood pressure recordings. Ultrasound guided radial microneurography was then performed. Multiunit MSNA recordings were obtained from the radial nerve of the non-dominant arm approximately 1/3 the length of the upper arm proximal

to the elbow. The neural activity was amplified, bandpass filtered (0.7-2kHz), rectified, and resistance/capacitance integrated (time constant 0.1 second)(Iowa Biosystems, Iowa, USA). Neural recordings were accepted 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). Subjects were fitted with the neck collar, after which they were asked to rest for approximately 10 minutes. The NP/NS protocol was performed according to Potts *et al* (Potts *et al.*, 1993) at rest and at steady state exercise HRs of 120 and 150 bpm, and during the final 15 min of 20 min inactive recovery from exercise in the 70° semi-recumbent position (Figure 1). Total time of exercise was 42.83 minutes (±1.19 min, SEM)

For protocols 1 and 2, reflex data for HR and MAP were analyzed and baroreflex curves were constructed according to Kent *et al* (Kent *et al.*, 1972). Saturation and threshold points were calculated according to McDowall and Dampney (McDowall & Dampney, 2006). Progressive gain curves were calculated for all the reflex curves as the first derivative of the baroreflex curve.

For protocol 2, MSNA was analyzed using WinCPRS (Absolute Aliens, Finland). Integrated neurograms were automatically peak detected and then manually inspected for accuracy of the detection. Signals were amplitude normalized to the quiet baseline and largest spontaneous peak occurring during a quiet non-stimulatory steady state period in both rest and recovery (0-100 arb. units). Nerve traffic is presented as both burst frequency (BF,bursts/minute) and as total activity (TA, nu/minute). TA is calculated by the product of BF and mean burst amplitude.

Two-factor ANOVA with repeated measures on stage (rest vs. recovery) were performed at $\alpha = 0.05$ such that $P<0.05$ was considered significant. When differences were detected, student Neuman-Keuls post hoc analysis was performed.

Results:

Demographic data are listed in table 1. Significant increases existed in HR for both semi-recumbent and upright during inactive recovery compared to rest and there was a significant decrease in HR during rest for the semi-recumbent group compared to the upright group. MAP was significantly lower during inactive recovery than rest in the semi-recumbent group (table 2).

Baroreflex Curve analysis:

No difference existed in any reflex characteristic parameter for MAP except for the well established resetting of the OP pressure up and to the right in a workload dependant manner (Fadel & Raven, 2012). Resetting up and to the right in a workload dependant manner also was shown for the carotid-cardiac baroreflex; however, significant differences between rest and recovery did exist. These differences are shown in figure 2A and table 2. There was an increase ($P<0.05$) in both semi-recumbent and upright exercise OP pressure heart rate (OPHR) during inactive recovery compared to rest and an increase ($P<0.01$) in OPHR in the upright compared to semi-recumbent. There was an increase ($P<0.05$) in the center point HR in both semi-recumbent and upright from rest compared to inactive recovery. Within inactive recovery there was an increase ($P<.05$) in the center point HR in semi-recumbent compared to upright.

The threshold and saturation pressures and gains of the baroreflex function curves during rest and recovery are listed in table 2 and summarized in figure 2. The threshold point pressure HR was lower ($P<0.01$) during rest in the semi-recumbent group vs. upright and was higher

(P<0.01) during inactive recovery compared to rest for both the upright and semi-recumbent groups. Saturation point pressure HRs were no different for rest upright, rest semi-recumbent and inactive recovery upright; however, the inactive recovery semi-recumbent HR at the saturation point pressure was higher (P<0.05). The HR response range was greater (P<0.05) during recovery in both upright and semi-recumbent. In addition, OP-Sat range was greater (P<0.05) in inactive recovery compared to rest for both upright and semi-recumbent, but less (P<0.01) in the semi-recumbent vs upright in both rest and recovery. The OP-Thr range HR was greater (P<0.01) in the semi-recumbent inactive recovery than semi-recumbent rest.

Gain curves were calculated from the baroreflex curves and maximal gain (Gmax) and OP gains were calculated for both HR and BP reflexes (figure 3). There was no difference in the Gmax or OP gain between rest and inactive recovery in the MAP curve (P>0.05). However, the HR Gmax was lower (P<0.05) in the semi-recumbent compared to upright in both rest and inactive recovery and the HR Gmax was higher (P<0.05) during inactive recovery compared to rest for both upright and semi-recumbent (table 2).

MSNA Analysis:

Muscle sympathetic nerve activity was successfully recorded throughout semi-recumbent cycling exercise and inactive recovery in 6 subjects. Both burst frequency and total activity were significantly increased from rest prior to exercise compared to inactive recovery, 18.5 ± 1.98 to 31.45 ± 5.39b/min (p=0.026) and 874 ± 88.0 to 1476 ± 246.1 nu/min (p=0.015), respectively (figure 5). Representative tracings are shown in figure 6.

Discussion:

The data obtained in this investigation provides new insights into the time course of the changes in sympatho-vagal balance during HR recovery and arterial baroreflex resetting from prolonged duration (43 to 58 min) moderate to heavy workload exercise. At the onset of inactive recovery central command is "switched off". However, the mechano- and metabo-receptor inputs from the respiratory muscles (Secher & Amann, 2012) and the metabolite activation of the EPR from the recovering skeletal muscles are slowly returning to resting activity (Rowell, 1993). During inactive recovery, both central command and its control of the vagal influence on HR would predictably be the same as at rest prior to exercise. Hence in the present study, we suggest that the reason the resting-recovery HR is higher than the resting HR prior to exercise (Figure 6) is a result of a diminishing but sustained increase in sympathetic activity resulting from activation of the EPR (Kaufman, 2012; Mitchell, 2013) of the respiratory and the recovering skeletal muscles

In studies using exercising human subjects and selective anesthetic (Fentanyl) blockade of respiratory muscle afferents (Secher & Amann, 2012) and epidural anesthesia (Lidocaine) of leg skeletal muscle afferents (Smith *et al.*, 2003) exercise HRs were reduced. In the epidural blockade studies carotid baroreflex control of HR function curves were reset down and to a lower OP pressure. In the present study, analyses of the CBR-HR reflex function curves between rest and recovery OP-Sat ranges (Figure 4) identify a greater range of response in the inactive recovery CBR curve compared to the pre-exercise resting CBR-HR curve. The OP-Sat range identified by the CBR-HR response to NS simulated hypertension identifies that during inactive recovery the HR at the OP pressure is higher than during pre-exercise rest and enables vagal activation to respond with greater affect to hypertensive stimuli (figure 4, table 2).

The centering point (CP) pressure of the pre-exercise resting CBR-HR reflex was no different from the inactive recovery CP; but due to a lower OP pressure and the sympathetically driven elevation in CP-HR the HR is higher on the curve. In the "Set Point" theoretical model of arterial baroreflex resetting at the point of exercise cessation, central command is immediately "switched off" and the "Set Point" reference pressure of the arterial baroreflex is reset to the resting OP pressure. Consequentially, the vagal activity rapidly dominates the sympatho-vagal balance controlling the HR, thereby returning the HR towards resting values. As noted above we propose that the reason for a greater recovery heart rate, compared to resting conditions, is an elevated maintenance of sympathetic outflow during recovery from prolonged exercise (figs. 2 & 5) due to increased EPR activity during the recovery. The apparent discrepancy between our findings and those of Halliwill's group (Halliwill *et al.*, 2013) can be explained by differences in posture (supine versus semi-recumbent) and time of recordings (30 versus 60 mins). Halliwill *et al.*'s report (Halliwill *et al.*, 1996) of decreased MSNA at 60 min of recovery were based on nerve recordings performed in the supine position, a position which reduces central MSNA outflow, because of loading of the cardiopulmonary baroreceptors (Charkoudian *et al.*, 2004), compared to the semi-recumbent recovery postures employed in the present investigation.

Changing posture affects the cardiovascular responses to exercise (Ray *et al.*, 1993; Rowell, 1993). Ogoh reported that increasing cardiopulmonary baroreceptor loading either by increased pedaling frequency or changing from upright to supine exercise decreases the steady state blood pressure (Ogoh *et al.*, 2007). In protocol 2 with subjects in semi-recumbent position, the cardiopulmonary baroreceptors (CPBRs) were partially unloaded at the end of exercise due to decreased muscle pump activity and venous return; the inhibitory drive was removed from the central outflow of MSNA resulting in increased peripheral MSNA (figures 5 & 6).

In protocol 1 with the subjects in the upright position we were limited by the inability to record MSNA during upright cycling due to lack of positional stability. However, the recovery MSNA was expected to be greater than that obtained during the semi-recumbent recovery (Figures 5 & 6), where the CPBRs would be unloaded to a greater degree. We suggest that the reductions in CBV during semi-recumbent and upright recovery result in reductions in CPBRs load and their mediated inhibition of MSNA. This, along with the lasting influence of EPR activation on increasing MSNA maintained elevated HRs during recovery. This suggestion is compatible with the findings of the pharmacological blockade studies of Savin et al. (Savin *et al.*, 1982). During resting-recovery the upright and semi-recumbent postures did not affect steady state HR or Gmax of the CBR-HR function curves. However, when compared to rest prior to exercise the HRs and Gmaxs were significantly higher (table 2, Figure 2A). We suggest that the differences in the HRs and Gmaxs of the CBR-HR reflex function curves indicate an increase in sympathetic drive to the heart because of unloading of the CPBRs and the arterial baroreflex compensation for the recovery peripheral vasodilatation induced by nitric oxide, increased blood pH and histamine receptor stimulation (Halliwill *et al.*, 2013).

Perspective:

This study confirms that increases and decreases in CBV loads sensed by the cardiopulmonary baroreceptors have a significant role in the baroreflex responsiveness during recovery from cycling exercise. The data provides important information in identifying mechanisms involved in post exertional bradycardia and syncope. In patient populations that have impaired arterial baroreflex control of blood pressure, age related stiffening of the arteries, and/or populations that suffer from severe post exercise induced syncope, recovery strategies that maintain heart rate and cardiac output will enable such populations to exercise with very little

risk. No load recumbent cycling during recovery from exercise might be a reasonable measure to ensure that there is no reflex induced bradycardia which could lead to syncope.

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Figure legends

Figure 1 Timeline for the experimental exercise protocol for both study 1 and 2. The recovery section is split into 2 sections. Active reduced workload recovery and inactive inactive recovery which the last 15 minutes is dedicated to building baroreflex curves.

Figure 2 A) Carotid-cardiac baroreflex curves. Solid lines represent resting data and dashed lines represent recovery. Semi-recumbent is black and upright is gray. B) Carotid-vasomotor baroreflex curves. Solid lines represent resting data and dashed lines represent recovery. Semi-recumbent is black and upright is gray. All data were analyzed by two way repeated measure ANOVAs and Neuman-Keuls post hoc analysis (*P<0.05, Rest vs Recovery; †P<0.05, Upright vs Semi-recumbent). SR, Semi-recumbent; UR, Upright; HR, Heart Rate; OP, Operating Point Pressure.

Figure 3 A) Carotid-cardiac baroreflex gain curves. Solid lines represent resting data and dashed lines represent recovery. semi-recumbent is black and upright is gray. B) Carotid-vasomotor baroreflex gain curves. Solid lines represent resting data and dashed lines represent recovery. semi-recumbent is black and upright is gray. All data were analyzed by two way repeated measure ANOVAs and Neuman-Keuls post hoc analysis (*P<0.05, Rest vs Recovery; †P<0.05, Upright vs Semi-recumbent). SR, Semi-recumbent; UR, Upright; HR, Heart Rate; OP, Operating Point Pressure.

Figure 4 Vagal influence on HR: Rest vs Recovery and Semi-recumbent vs Upright. Dark gray bars are semi-recumbent and light gray bars are upright. OP-Thr values are the rise in HR caused by a hypotensive stimulus and represent the ability to reflexively withdraw the vagal input. OP-Sat values are the decrease in HR caused by a hypertensive stimulus and represent the

ability to reflexively increase vagal input. All data were analyzed by two way repeated measure ANOVAs and Neuman-Keuls post hoc analysis (*P<0.05, Rest vs Recovery; †P<0.05, Upright vs Semi-recumbent). SR, Semi-recumbent; UR, Upright; HR, Heart Rate; OP-Sat, Change in HR from the operating point pressure to the saturation point pressure; OP-Thr, Change in HR from the operating point pressure to the threshold point pressure.

Figure 5 Muscle sympathetic nerve activity recorded during rest and recovery in the semi-recumbent position. Blue bars indicate quantification of nerve traffic as burst frequency (bursts/min) and red bars indicate it in total activity (nu/min). There is a significant increase in nerve traffic during recovery with both methods of quantification. All data were analyzed by one way repeated measure ANOVAs and Neuman-Keuls post hoc analysis (*P<0.05, Rest vs Recovery). BF, Burst Frequency; TA, Total Activity; nu, normalized units.

Figure 6 Representative neurogram of one subject in both the resting and inactive recovery stage of the protocol. Time length of the tracing is 30 seconds.

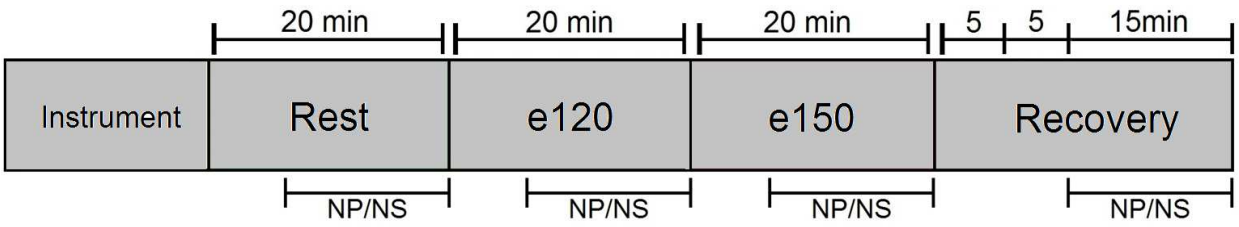
	Protocol 1 Upright		Protocol 2 Semi-recumbent	
	Mean	SEM	Mean	SEM
Age (years)	24.4	± 1.16	25.5	± 0.88
Height (cm)	179	± 2.47	176.8	± 2.76
Weight (Kg)	79.8	± 3.33	74.9	± 3.67
Resting HR (bpm)	71.2	± 2.00	63.5	± 1.61†
Resting MAP (mmHg)	90.1	± 2.52	96	± 2.06
e120 (watts)	101.3	± 12.77	67.2	± 6.58
e150 (watts)	158.7	± 16.12	128.5	± 11.69

Table 1. Demographics for the two protocols. Data are listed as means and standard error of the means (SEM). (†P<0.05, Upright vs Semi-recumbent)

	Rest		Recovery	
	Upright	Semi-Recumbent	Upright	Semi-Recumbent
OPHR (bpm)	73.0 ± 2.57	63.4 ± 1.74†	86.7 ± 3.00*	87.6 ± 1.94*
MAP (mmHg)	91.7 ± 2.76	96.5 ± 2.16	90.1 ± 2.49	90.7 ± 2.01*
Max Gain HR (-bpm/mmHg)	0.64 ± 0.09	0.37 ± 0.06†	0.90 ± 0.07*	0.61 ± 0.07*†
Gain OP HR (-bpm/mmHg)	0.46 ± 0.08	0.29 ± 0.07	0.58 ± 0.10	0.49 ± 0.04*
Threshold HR (bpm)	78.9 ± 2.94	68.1 ± 2.31†	95.5 ± 2.72*	97.7 ± 2.18*
Saturation HR (bpm)	58.6 ± 3.78	58.1 ± 1.74	59.4 ± 5.30	74.0 ± 4.04*†
Response Range HR (bpm)	22.5 ± 3.75	11.2 ± 1.24†	40.1 ± 5.40*	26.3 ± 3.80*†
OP-Sat (bpm)	-14.4 ± 3.10	-5.3 ± 0.96†	-27.3 ± 2.96*	-14.0 ± 5.08*†
OP-Thr (bpm)	5.9 ± 1.63	4.9 ± 1.32	8.8 ± 2.00	10.1 ± 1.84*

Table 2. Results from protocols 1 (Upright) and 2 (Semi-recumbent). Values are means ± SEM. All data were analyzed by two way repeated measure ANOVAs and Neuman-Keuls post hoc analysis (*P<0.05, Rest vs Recovery; †P<0.05, Upright vs Semi-recumbent). HR, Heart Rate; MAP, Mean Arterial Pressure; OP, Operating Point; OP-Sat, Change in HR from the operating point pressure to the saturation point pressure; OP-Thr, Change in HR from the operating point pressure to the threshold point pressure.

Figure 1


20 min
20 min
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Instrument
Rest
e120
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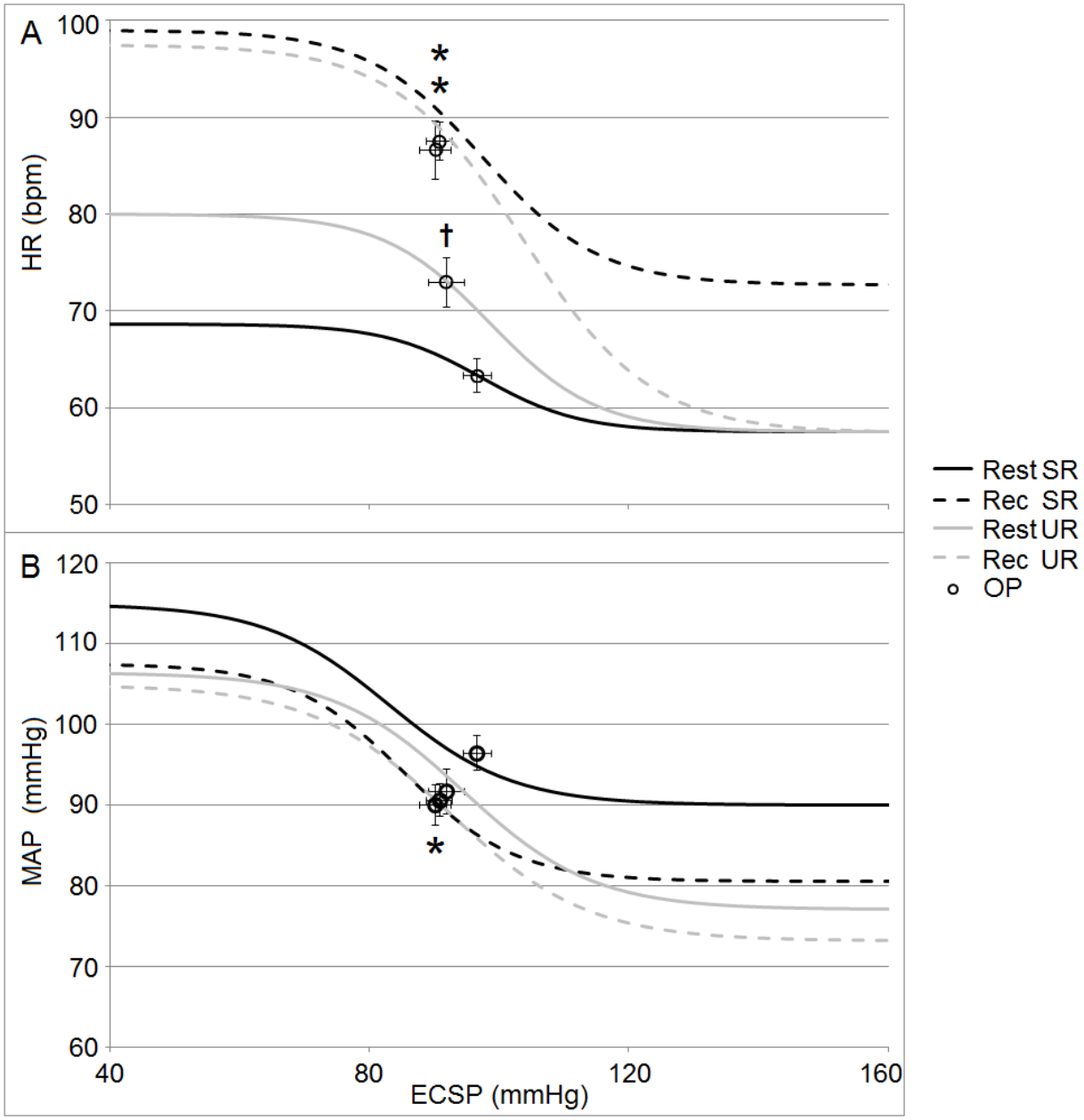
Recovery
NP/NS
NP/NS
NP/NS
NP/NS

Figure 2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B
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MAP (mmHg)
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ECSP (mmHg)
Rest SR
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Rest UR
Rec UR
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Figure 3

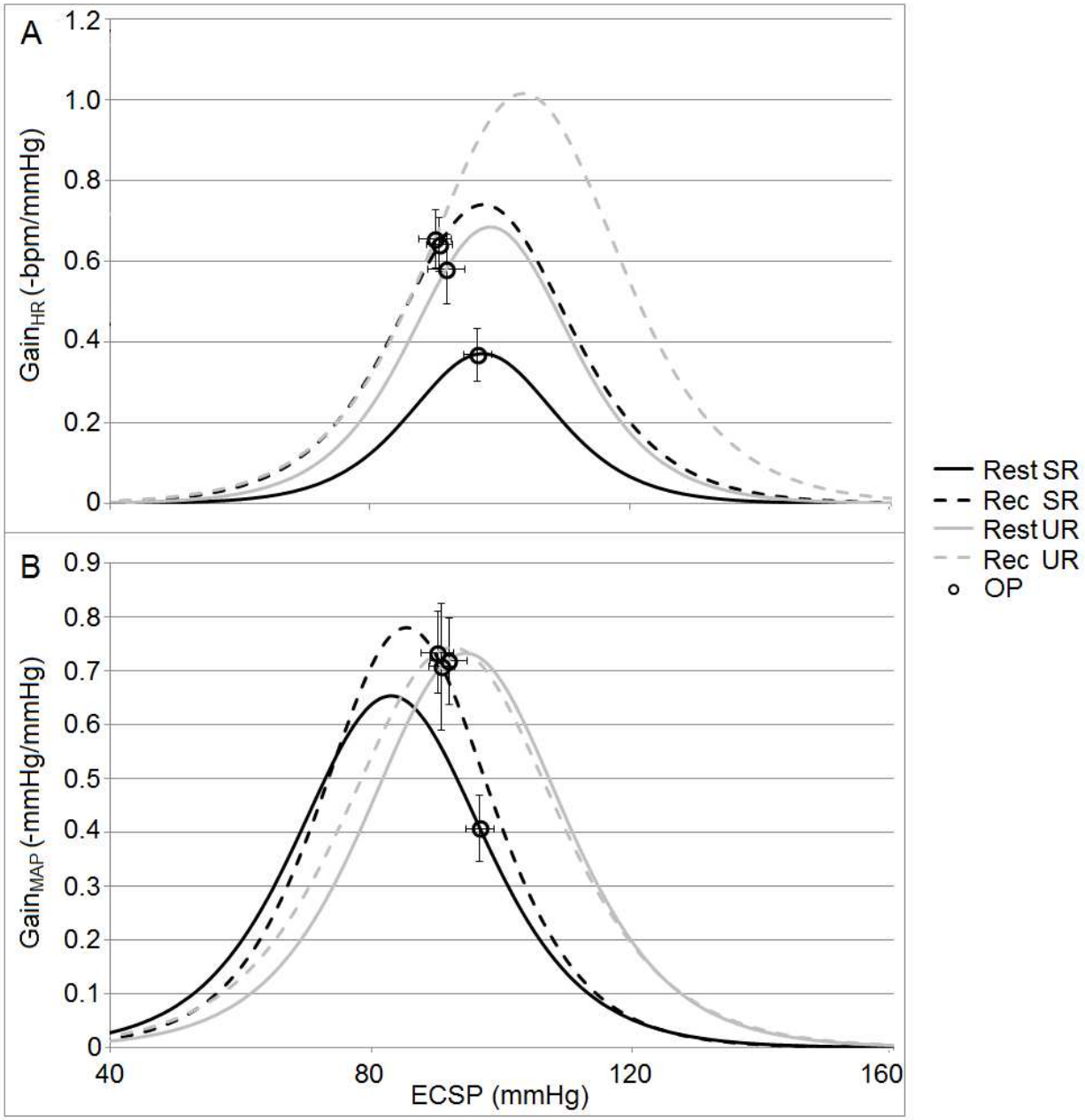
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Figure 4

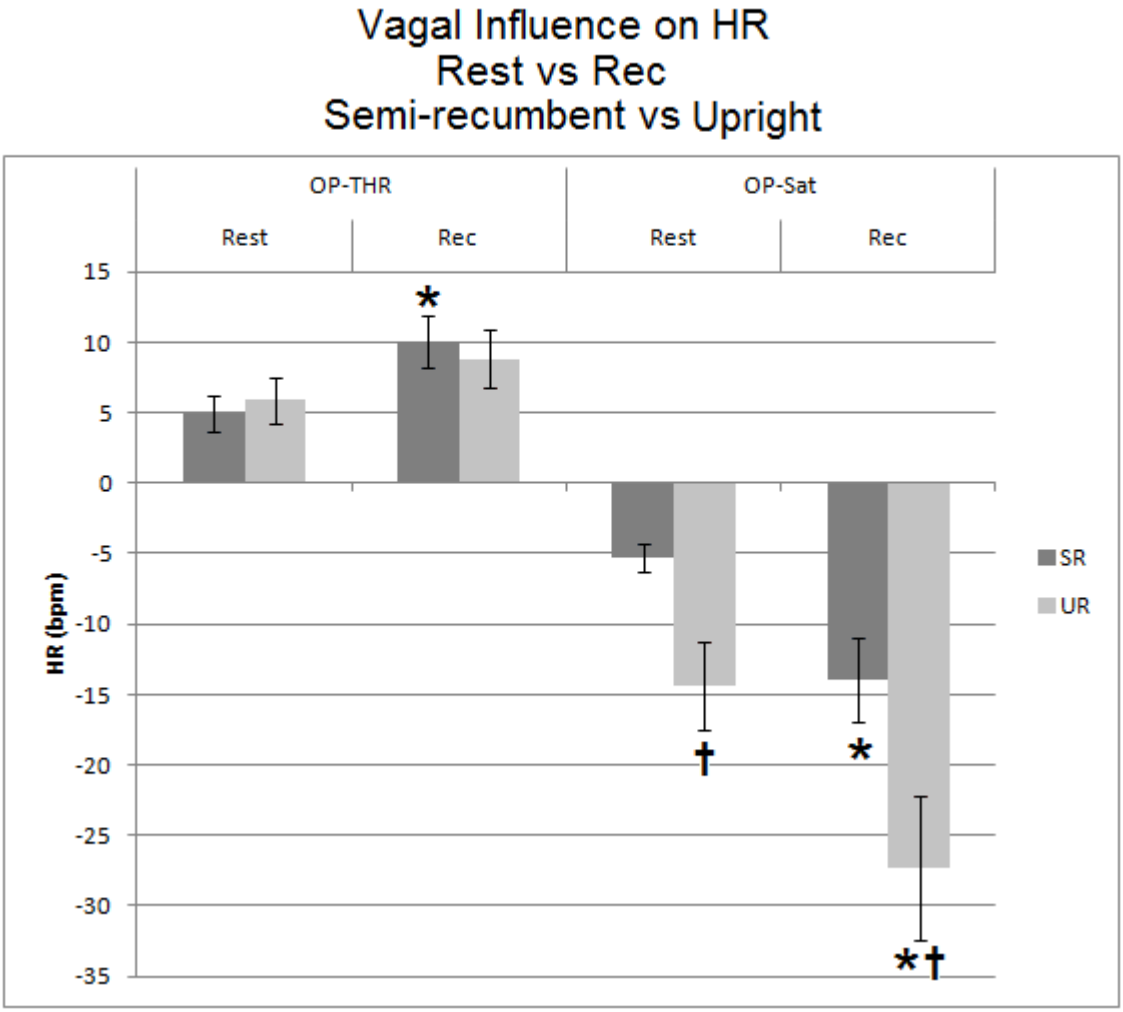
Vagal Influence on HR
Rest vs Rec
Semi-recumbent vs Upright
OP-THR
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Figure 5

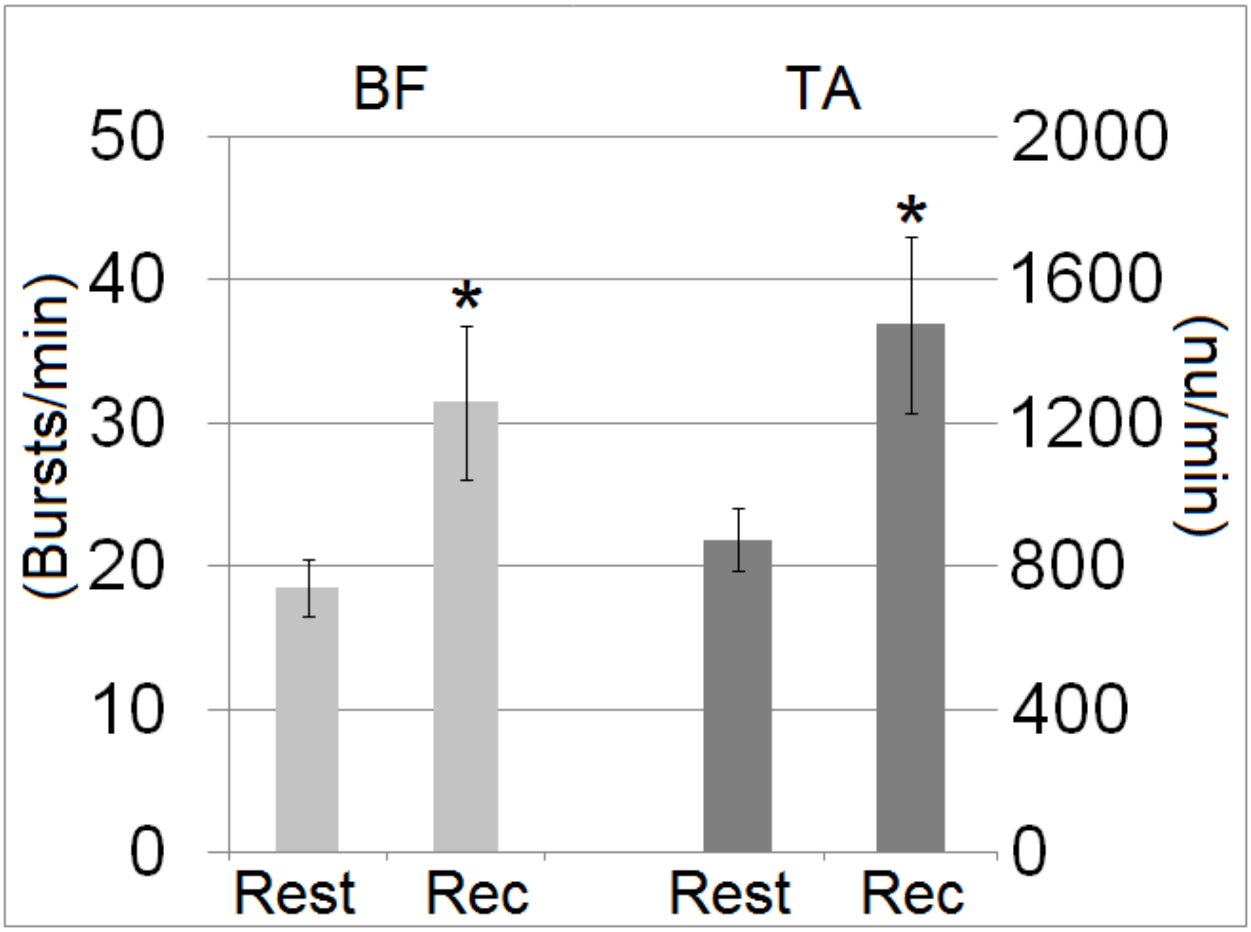
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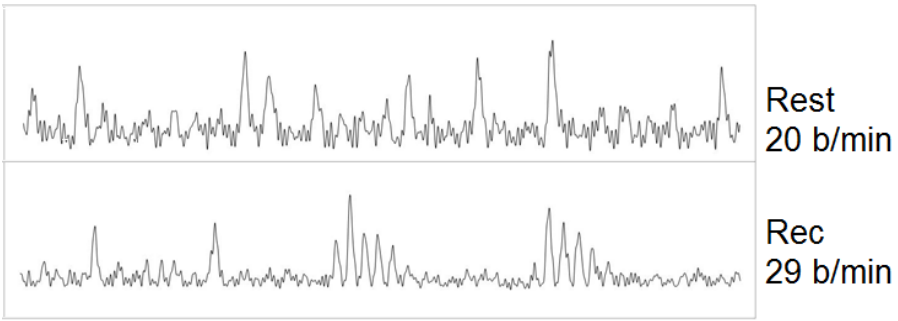
Figure 6



CHAPTER IV

Cardio-respiratory interactions immediately following dynamic leg cycling: influences of the muscle pump

Daniel W. White, Gilbert Moralez, Victoria L. Kay, Wendy L. Eubank,

Peter B. Raven

Abstract:

Changes in cardiorespiratory function during the moments immediately following prolonged exercise have yet to be fully explored. The mode of recovery affects cardiovascular function during recovery, but the change from active to inactive recovery has not been fully elucidated. We hypothesized that the cessation of muscle pump activity and unloading of the cardiopulmonary baroreceptors during early recovery would allow for exacerbated oscillations in cardiovascular variables that are neurally mediated. To test this hypothesis, subjects performed two-legged cycling exercise at moderate and heavy workloads and then performed active followed by passive recovery. Data analysis was done in the minute prior to cessation of pedaling and the first minute of inactive recovery. Spontaneous baroreflex sensitivity was significantly greater during inactive recovery compared to active. Also, the sensitivity of the respiratory/blood pressure and the respiratory/heart rate interactions were increased during inactive recovery compared to active recovery. We conclude that the respiratory-induced oscillations in hemodynamics during recovery from exercise are dependent on muscle pump activity and central blood volume.

Introduction:

Central command (CC), the exercise pressor reflex (EPR), the arterial baroreflexes (ABR) and the cardiopulmonary baroreflexes (CPBR) interact to regulate blood pressure (BP) and heart rate (HR) responses to dynamic exercise (Fadel & Raven, 2012; Mitchell, 2013). However, the role of these neural mechanisms in regulating HR recovery from dynamic exercise remains a question (Coote, 2010). There is a fine reciprocal balance between tonic sympathetic activity and vagal modulation. This balance is modulated by loading of the cardiopulmonary baroreceptors (Levy, 1971; Pawelczyk & Raven, 1989; Rowell, 1993; Potts *et al.*, 1995; Charkoudian *et al.*, 2004; Fadel & Raven, 2012). The cardiopulmonary baroreceptors are mechano- and metabo-receptors that sense mechanical and metabolic cardiac wall stresses related to central blood volume (CBV) and transmit c-fiber afferent neural information to the nucleus tractus solitarius (NTS) and play a major role in regulating HR (Mark & Mancia, 1983; Collins & DiCarlo, 1993). During dynamic leg exercise, the cardiopulmonary baroreceptors sense the increase in cardiac wall stress due to the muscle pump increasing venous return and CBV. The increase in CBV results in decrease in the responding range of the baroreflex control of both HR and BP (Potts *et al.*, 1995). In addition, muscle sympathetic nerve activity (MSNA) is also decreased (Charkoudian *et al.*, 2004). What has not been fully elucidated is the influence of CBV on the autonomic neural control of HR and BP during recovery from prolonged exercise (Coote, 2010).

During early recovery from exercise, respiratory tidal volumes (V_t) are exaggerated to facilitate gas exchange and to re-establish acid-base balance (Wasserman, 2005). Eckberg *et al.* reported that MSNA was more excitable during the expiratory phase of respiration (Eckberg *et al.*, 1985) and concluded this was a result of central integration of respiratory stretch receptors

and baroreceptor afferent nerve traffic within the NTS. Another interpretation of their findings may be that during respiration the oscillatory decreases and increases in CBV and, therefore, unloading and loading of the cardiopulmonary baroreceptors resulted in oscillatory and modulatory inhibition of arterial baroreflex integration of HR, BP and MSNA within the NTS. Ray et al. having subjects peform one-legged knee extension in the upright position, while measuring MSNA in the contralateral leg reported a decrease in MSNA during exercise. The decreased MSNA continued through the leg kicking exercise until the onset of inactive recovery, at which time MSNA exceeded the resting MSNA (Ray *et al.*, 1993). However, when the subjects performed the same exercise in the supine position the MSNA during the inactive recovery was no different from the pre-exercise rest. Furthermore, the MSNA recorded in the supine position was no different from that recorded while exercising in the supine or upright positions. These results call into question whether the one legged kicking exercise induced a greater loading of the cardiopulmonary baroreceptors than supine rest. In contrast, Halliwill *et al*. have reported a decrease in MSNA recorded one hour after prolonged cycling exercise in the supine position compared to one hour of MSNA recordings during supine rest (Halliwill *et al.*, 1996a).

In a recent study in our laboratory addressing a separate question (White *et al.*, 2014), it was observed that the magnitude of HR change for a given depth of respiration changed from active recovery to inactive recovery. Therefore, we hypothesized that loading of the cardiopulmonary baroreceptors with muscle pump activity decreases the magnitude of respiratory modulation of HR, BP, and MSNA during recovery from prolonged exercise. We tested this hypothesis by having subjects perform prolonged cycling exercise followed by active recovery with reduced load cycling followed by inactive recovery. The differences in magnitude

of the changes in HR, BP or MSNA for the same depth of respiratory tidal volume (Vt) between active and inactive recovery were considered to be a result of muscle pump loading of the cardiopulmonary baroreceptors.

Methods:

13 healthy human subjects (3 female) aged 25.5 years (±0.88) volunteered for the study. None of the volunteers had a history of heart disease, respiratory disease and all were non-tobacco users. On an orientation/familiarization day, each subject completed the institutional review board (IRB) approved consent paperwork and a health history questionnaire (IRB#2010-058). Then each subject underwent a physical examination and graded exercise test. The physical examination included 12-lead electrocardiogram and automated BP auscultation (Suntech Tango+, Morrisville, NC) in both the sitting position and upon standing. Upon completion of the physical examination the subjects mounted a semi-recumbent cycle ergometer (Intellifit Psycle, Bedford, Texas) and were instrumented for 3-lead ECG (HP 78342A) and beat to beat BP measurement with the Finometer (Finapres Medical Systems, Amsterdam). Each subject started at a very light workload and was asked to maintain a pedaling cadence of 60 rpm as the workload was step increased every 2 minutes. The graded exercise test was concluded when the subject was unable to continue despite strong verbal encouragement or was no longer able to maintain 60 rpm. Each subjects HR at each workload was recorded and an individual HR/workload regression line was used to determine each subject's workloads necessary to attain HR of 120 (e120) and 150 (e150) beats per minute.

No less than 2 days later the subjects returned for the full exercise protocol. Subjects were asked to mount the semi-recumbent cycle ergometer and ECG and BP were recorded. Subjects were also instrumented for impedance pnuemography (Minnesota Impedance

Cardiograph model 304B, Minnesota, USA) to measure depth of respiration. Ultrasound guided radial nerve microneurography was then performed. Multiunit MSNA recordings were obtained from the radial nerve of the non-dominant arm approximately 1/3 the length of the upper arm proximal to the elbow. The neural activity was amplified, bandpass filtered (0.7-2kHz), rectified, and resistance/capacitance integrated (time constant 0.1 second) (Iowa Biosystems, Iowa, USA). Neural recordings were accepted 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). Subjects were asked to start pedaling at a very low workload that was then slowly increased to a workload to induce a HR of 120bpm for 15 min. Then the workload was slowly increased to induce a steady state HR of 150bpm for 15 min. For recovery, the workload was reduced until the HR reached 120 bpm steady for 2 minutes and then pedaling was stopped. Subjects then recovered without moving for 15 minutes. Due to loss of nerve signal and erratic respiratory patterns, only data from 6 volunteers could be analyzed. Total time of exercise was 42.83 minutes (±1.19 min, SEM) (Figure 1).

Heart rate, BP and MSNA were analyzed using WinCPRS (Absolute Aliens, Finland). Integrated neurograms were automatically peak detected and then manually inspected for accuracy of the detection. Signals were amplitude normalized to the quiet baseline and largest spontaneous peak occurring during a quiet non-stimulatory steady state period (0-100 arb. units).

Spontaneous baroreflex sensitivity was analyzed by the sequence method (Bertinieri *et al.*, 1985). Sets of three consecutive beat to beat changes in systolic arterial pressure were associated with changes in R-R interval in the same direction at a 1 second delay.

Frequency analysis was performed on respiratory rhythms from the impedance pnuemography signals. Then spectral coherence was calculated against HR, mean arterial pressure and integrated neurogram at the frequency of respiration.

Impedance pnuemography signals were manually inspected during the last minute of active recovery and the first minute of inactive recovery. The differences in the peak amplitude of the signal from the mean of the signal were recorded and compared to the corresponding peak changes in HR, mean arterial pressure and MSNA.

One-way repeated measures ANOVA was performed on mode of recovery (Active vs. Inactive) at $\alpha = 0.05$. When significance was detected Student Neuman-Keuls post hoc analysis was performed. P values of <0.05 indicate statistically significant differences in mean values.

Results:

Average respiration rates in the 1 minute prior to and 1 minute following cessation of active recovery were 28.0 ± 1.4 and 22.4 ± 2.4, respectively ($P<0.05$). Values of coherence of respiration to HR, MAP, and MSNA are shown in table 1. Thoracic impedance (Zo) was significantly higher during inactive vs. active recovery (57.1 vs. 55.8, P=0.02). MSNA was increased during inactive recovery (150.1%, P=0.15; Figure 3). There was no difference in the change from mean to peak V_t between active and inactive recovery. Responses in MAP to inhalation were increased during inactive recovery (-7.58 ± 1.25 vs. -5.22 ± 1.35, P=0.289); responses in HR to inhalation were also increased during inactive recovery (5.85 ± 1.56 vs. 3.67 ± 0.70, P=0.329; Figure 4). Slope of the V_t /MAP relationship was more negative during inactive recovery (-11.32 vs. -3.95 mmHg/fold increase); slope of the V_t /HR relationship was more positive during inactive recovery (8.67 vs. 2.78 bpm/fold increase). Spontaneous

baroreflex sensitivity was significantly increased during inactive recovery for the Up-Up reflex (2.58 fold, $P<0.05$) and tended to be increased for the Down-Down reflex (1.53 fold, P=.107; Figure 5).

Discussion:

The data identifies that a strong coherence exists between respiratory rhythm and oscillations in HR, BP, and MSNA (table 1), but the magnitude of the changes in the variables are greatly dependent on whether or not the muscle pump is active or inactive, see figure 2. This finding provides additional evidence that the C-fiber afferents, generally termed cardiopulmonary baroreceptors sense the changes in ventricle wall transmural pressures and its metabolite milieu (Paintal, 1973) and are involved in reflex regulation of arterial blood pressure (Fadel & Raven, 2012).

In the present study we used Impedance pnuemography to monitor changes in thoracic impedance ($TI=Z_0$) associated with the increases and decreases in central blood volume (CBV) resulting from pulmonary inspiration and expiration, respectively. In the transition from active recovery there is a marked increase in Z_0 indicating a decrease in CBV. This decrease in CBV identifies an unloading of the cardiopulmonary baroreceptors which has been identified to result in an increase in MSNA (Charkoudian *et al.*, 2004) see figure 2 and figure 3. In addition to the unloading of the cardiopulmonary baroreceptors being associated with the cessation of the pedaling exercise the magnitude of the respiratory oscillations identified by increases in Vt markedly affect BP and HR fluctuations (Figure 4). Also, at the time when cessation of pedaling occurs and the decrease in CBV is accentuated, the data identify an increase in spontaneous baroreflex sensitivity (Figure 5). Halliwill et al. reported similar findings (Halliwill *et al.*, 1996b). From our observations, we conclude that the increased fluctuations in HR that occur at

the cessation of exercise are directly linked to respiratory induced fluctuations in arterial pressure and baroreflex control of HR, schematically presented in figure 6.

The models of neural interactions presented in figure 6 are a proposed integrated mechanism to explain the data during active and inactive recovery. During active recovery, muscle pump activity along with inspiratory increases in CBV load the cardiopulmonary baroreceptors and.inhibit sympathetic outflow (Eckberg *et al.*, 1985). However, the increased inhibition of sympathetic outflow only resulted in a relatively small decrease in arterial BP. Once the muscle pump activity is stopped the CBV will decrease and when combined with the inspiratory inhibition of sympathetic activity will result in the much greater drop in arterial BP observed in the present investigation. The drop in arterial BP recorded in the present study was probably a result of metabolite induced vasodilatation in the active muscles (Halliwill *et al.*, 2013) and pooling of blood in the legs during resting-recovery SR and UR postures. Subsequently, the drop in arterial BP results in the larger baroreflex mediated increase in HR attributed to the: i) greater baroreflex sensitivity; and ii) greater decreases in arterial pressure observed in the present study. Respiratory coupling to HR was first described by Hering and Breuer (Hering & Breuer, 1868) as a reflex to stretch of mechanoreceptors in the lungs being inhibitory to vagal outflow. In our current observations, the temporal relationships between inhalation and increases in HR seem to be too slow to explain the HR responses observed, though this doesn't exclude the possibility of summation of Hering-Breuer reflex and arterial baroreflex.

The afferent nerve traffic, during inactive recovery, from the mechano-receptors and metabo-receptors located within the respiratory muscles may also be causing an exaggerated un-opposed increase in tonic sympathetic nerve activity and causing the larger magnitude BP

fluctuations observed. The greater negative slope (figure 4) of the V_t/MAP relationship and the greater positive slope of the V_t/HR relationship observed at the transition from active to inactive recovery indicates an increase in the sensitivity of the neural reflex arc linking respiration to cardiovascular function. However, because the transition from active to inactive recovery involves the cessation of the muscle pump, we conclude that the integration of the mechanical displacement of blood to and from the thorax by the muscle pump during active recovery suppresses the amplitude of oscillations in neural control of HR, BP, MSNA and TI evoked by the respiratory pump during inactive recovery, see figure 2.

Perspective:

The clinical value associated with advanced understanding of the autonomic nervous system is incalculable. Delayed recovery in HR following exercise is a strong predictor of mortality (Cole *et al.*, 1999; Desai *et al.*, 2001b) and that the cardioacceleration efficiency is linked to cardiodeceleration (Desai *et al.*, 2001a). Slow HR recovery is associated with the metabolic syndrome (Kizilbash *et al.*, 2006); however, data has been lacking in the area of the neural control of HR during recovery in order to establish a reliable and sensitive measure of autonomic dysfunction (Borresen & Lambert, 2008).

The magnitude of oscillations of HR during passive recovery were greater both in the positive and negative directions which is important especially in the case of a person with post-exertional syncope where a possible threshold bradycardia could potentially exist. Post-exertional syncope may be the only predictor of sudden cardiac death in young athletes (O'Connor *et al.*, 1999). The fluctuations of HR during active recovery were biased in the positive direction which might lead to preventative interventions in persons with a history of

post-exertional syncope. Instead of telling people not to exercise, it could be prescribed that mounting a recumbent cycle for low load recovery would be beneficial.

Characterization of normal cardiovascular function in the moments following a heavy workout may provide insight into diagnosis of certain pathologies not seen during rest or late recovery.

Limitations:

There is a very short period of time in which data can be collected and conclusions can be made between active and inactive recovery before the active and inactive HRs are significantly different. These differences could lead to inaccurate conclusions about the amount of muscle sympathetic outflow which is why we chose to present the data as total activity. This measure is less sensitive to changes in HR because it measures the total amount of time in which nerve activity is occurring over the period of a minute, not just how many burst occurrences happened in the same minute which is dependent on HR. The choice was made not to express MSNA in burst incidence or total MSNA because the amount of time to normalize to 100 heart beats can be drastically different from one subject to another. Hence, we chose to keep our analysis to a static time period for this reason. Also the collection of MSNA during cycling is a daunting task and therefore only 3 subject's signals were able to be analyzed in both active and inactive recovery. Therefore the power of the statistic was low ($1-\beta=0.219$) and increases the possibility of a type II error.

Conclusion:

We are the first to analyze MSNA during immediate recovery after prolonged two-legged cycling exercise. Loading of the CPBR due to muscle pump activity causes decreases in arterial

baroreflex sensitivity and the respiratory induced oscillations in the cardiovascular functional variables of HR, MAP, MSNA and TI. .

Acknowledgements:

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Figure legend

Figure 1 Schematic diagram of the exercise protocol timeline. Inset is a representation of the analysis period 1minute before to 1 minute after cessation of active recovery. e120, exercise at a HR of 120 beats per minute; e150, exercise at a HR of 150 beats per minute.

Figure 2 Continuous tracing of a representative subject from active to inactive recovery. Fluctuations in HR (bpm), MAP (mmHg) and MSNA (au) are all associated with changes in TI amplitude. Cut-outs are 30 second scaled tracings of TI and MSNA. HR, Heart Rate; MAP, Mean Arterial Pressure; MSNA, Muscle Sympathetic Nerve Activity; TI, Thoracic Impedance Pneumograph.

Figure 3 Percent change in total MSNA from active recovery to inactive recovery. MSNA, Muscle Sympathetic Nerve Activity.

Figure 4 Changes in MAP and HR for a given change in respiratory V_t. Diamonds represent changes during active recovery and squares represent changes during inactive recovery. The slope of the connecting lines may be interpreted as response sensitivity to V_t. MAP, Mean Arterial Pressure; HR, Heart Rate; V_t, Respiratory Tidal Volume.

Figure 5 Spontaneous baroreflex sensitivity. Columns represent the percent change in sensitivity from active (light gray) to inactive (dark gray). On the left is the response in R-R interval to increases in systolic arterial pressure (Up-Up) and on the left is the response in R-R interval to decreases in systolic arterial pressure. Data was analyzed using paired t-test. * Significant difference from active, $P<0.05$.

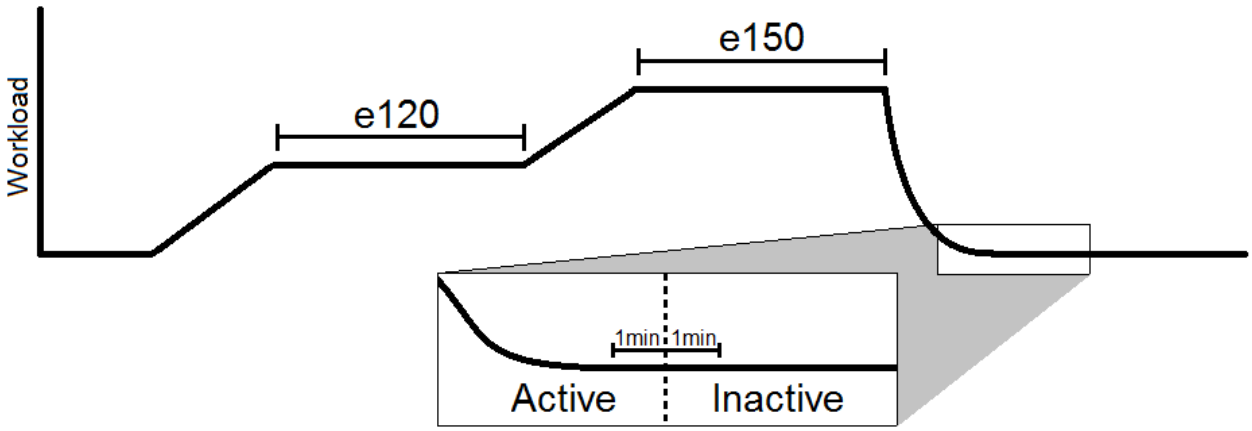
Figure 6 Schematic diagrams of the proposed neural interactions during active and inactive recovery following prolonged two legged dynamic cycling exercise. Stick arrows represent interactions. Open arrows represent approximate magnitude of responses to inhalation (left) and exhalation (right). Solid arrow indicates the relative state of arterial baroreflex sensitivity. CPBR, Cardiopulmonary Baroreflex; EPR, Exercise Pressor Reflex; SNA, Sympathetic Nerve Activity; BRS, Arterial Baroreflex Sensitivity; BP, Blood Pressure; HR, Heart Rate.

Table 1: Respiratory Frequency and Coherence

	Active	Inactive
Breath/min	27.6 ± 1.37	22.4 ± 2.43*
Thoracic Zo (Ω)	55.8 ± 4.08	57.1 ± 4.49*
HR coh	0.97 ± 0.01	0.98 ± 0.02
MAP coh	0.98 ± 0.01	0.97 ± 0.01
MSNA coh	0.84 ± 0.04	0.88 ± 0.06

Values of respiratory frequency and basal thoracic impedance. Power spectral coherence values at the respiratory frequency for each subject in HR, MAP and MSNA during active and inactive recovery. Values are mean and SEM. HR, Heart Rate; MAP, Mean Arterial Pressure; MSNA, Muscle Sympathetic Nerve Activity. * Significant difference from active, $P<0.05$.

Figure 1



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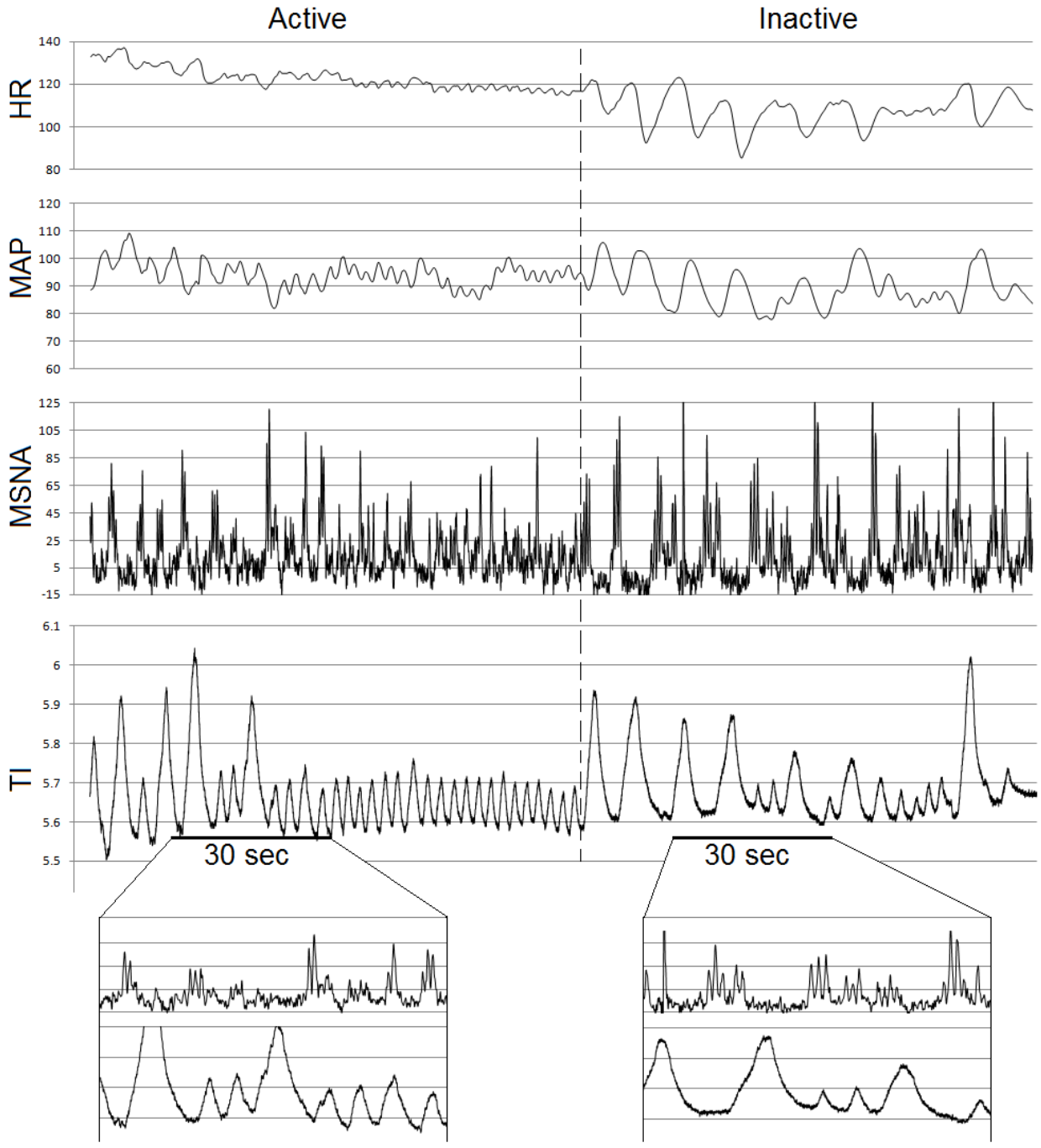
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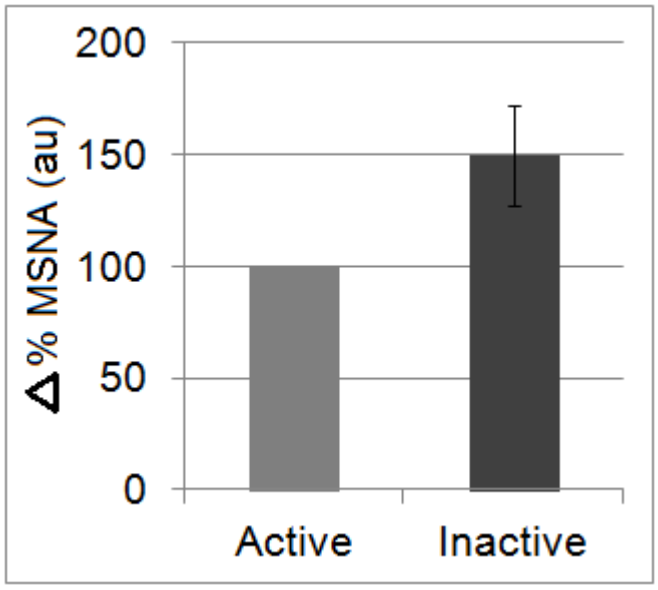

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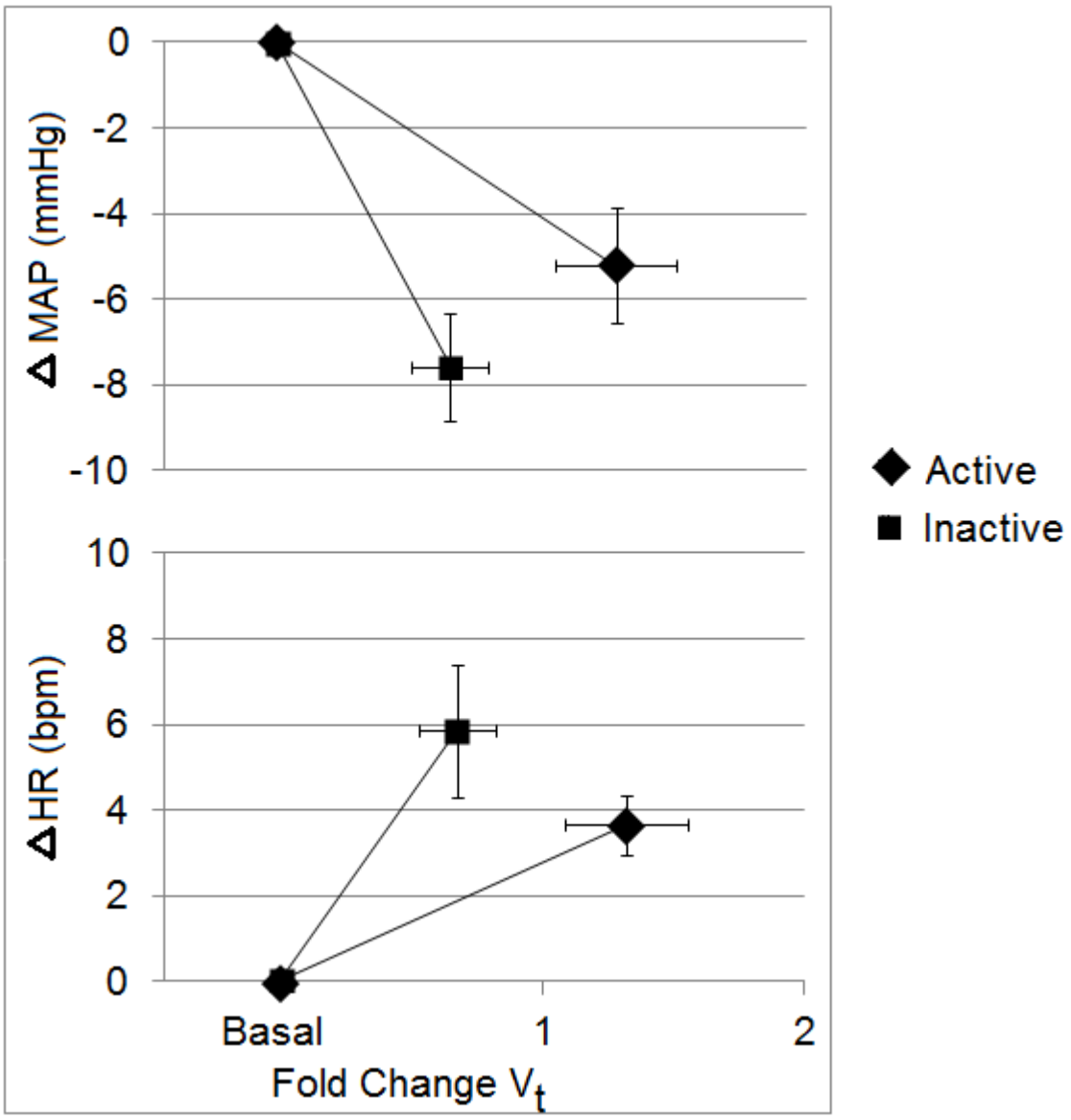
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Figure 5

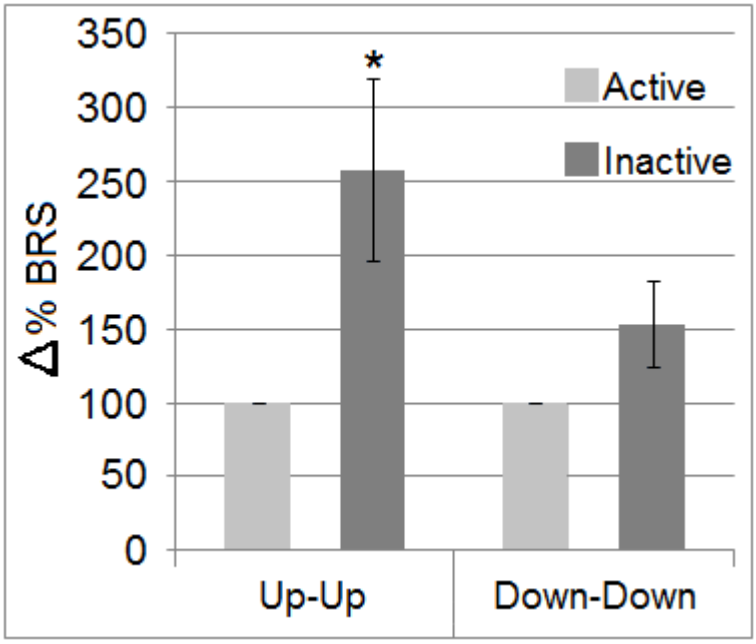
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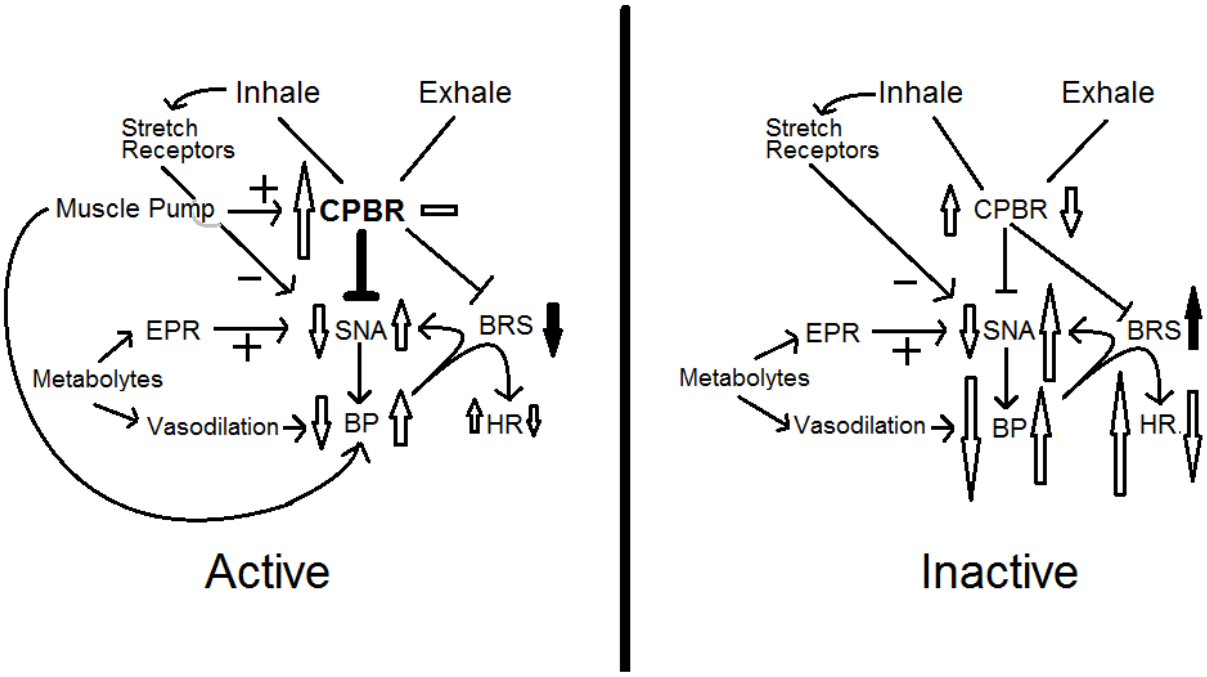
Figure 6


Inhale
Exhale
Stretch Receptors
Muscle Pump
CPBR
EPR
SNA
BRS
Metabolytes
Vasodilation
BP
HR
Active
Inhale
Exhale
Stretch Receptors
CPBR
EPR
SNA
BRS
Metabolytes
Vasodilation
BP
HR
Inactive

CHAPTER V

Conclusion

The results of these investigations within this dissertation have led us to a revitalized perspective on the concept of autonomic control of heart rate during exercise and recovery. Our overall findings indicate that the role of the cardiopulmonary baroreceptors have an immediate and strong inhibitory influence on the parasympathetic modulation of the reflex control of HR at the onset of and throughout exercise and recovery.

Our findings obtained through the use of newer techniques and advanced understanding of the autonomic nervous system, challenge the contemporary concept that; the increases in HR during a progressive increase in work load to maximal exercise test is a result of an initial vagal withdrawal from rest to a HR of 100 beats/min followed by sympathetic activation up to maximum. In contrast, our data points toward a previously reported concept of a dynamic balance between sympatho-vagal control of HR ranging from an 20:80 ratio of vagal dominance at exercise onset to an 80:20 ratio of sympathetic dominance at maximum exercise.

The investigation of baroreflex control during rest and inactive recovery identify that the loading and unloading of the cardiopulmonary baroreceptors, as a result of increases and decreases of CBV, modulates the arterial baroreflex control of HR by decreasing and increasing sympathetic activity, respectively. Orthostatic loading during rest prior to exercise onset lowered the sensitivity and response range of the arterial baroreflex. This decrease in sensitivity and response range was also found during resting-recovery from heavy exercise, but in contrast to

rest prior to exercise, recovery HR was no different based on orthostatic position. Furthermore, from our data, we conclude that the higher HR during recovery is due to increases in sympathetic nerve activity as a result of an activated exercise pressor reflex (EPR).

During resting-recovery there was no difference in the maximal gain (Gmax) of the carotid-vasomotor baroreflex. Interestingly, the arterial pressure at which the Gmax of the CBR-HR was located was no different between pre-exercise rest and resting-recovery in both the semi-recumbent and upright positions. From these findings we concluded that orthostatic challenges do not affect autonomic reflex control of blood pressure to the same degree as observed for control of HR. Hence we suggest that the reason for the elevated HR during resting-recovery was due to an increased sympathetic influence at the sino-atrial node of the heart.

Further analysis of the cardio-respiratory data recorded during the transition from active to inactive recovery identified the influence of respiration on arterial blood pressure regulation. During the transition from pedaling to no pedaling the TI measure increased identifying a decrease in CBV that resulted in a transition from a low gain to a high gain control system. Upon discontinuation of pedaling, the oscillatory fluctuations in BP and HR became greater and were proportional to the oscillations respiratory Vt. On closer analysis of the data, the respiratory induced fluctuations in BP appeared to be a direct result of inspiratory inhibition of MSNA associated with the respiratory pump loading of the cardiopulmonary baroreceptors.

Simultaneously with the respiratory inhibition of MSNA, residual metabolite induced vasodilation of the previously active skeletal muscles resulted in the greater decrease in BP observed during the resting-recovery compared to the pre-exercise resting BP. Because of the increase in the resting-recovery BP oscillations, a coherent oscillatory activation and deactivation of the arterial baroreflexes result in the observed increase HR oscillations. These findings

provide additional evidence that the oscillatory increases and decreases in CBV associated with respiration result in arterial baroreflex induced BP oscillations and subsequent 2nd order HR oscillations.

CHAPTER VI

Suggestions for future research

The research presented in this dissertation provided new insights into the role of the autonomic nervous system during recovery from prolonged heavy cycling exercise. However, as is often the case in the process of addressing the questions to advance our knowledge new questions arise. The following is a list of potential investigations for the better understanding of recovery following exercise.

I. Further investigations into the baroreflex control of muscle sympathetic nerve activity during heavy dynamic two-legged cycling need to be performed. Current research identifies with arm cycling a resetting of the carotid-MSNA baroreflex function curve up and to the right but preliminary data with leg cycling suggests that at low workloads the carotid-MSNA reflex is reset down and to the right. The question raised is "What is the mechanism by which this downward resetting occurs?"

II. The effect of long term physical training on the immediate responses to cessation of exercise needs to be explored along with age related influences. The same procedures used in this dissertation could be applied to a longitudinal repeated measures design. Cardiovascular plasticity with long term training could change the outcomes of the current investigations in a beneficial manner, and furthermore, identify age related changes in cardiovascular health.

III. After clarifying the physiological hemodynamic responses of cardiovascular control during recovery from exercise, it would be beneficial to address the many questions involving recovery from exercise of autonomic nervous system impaired patients.