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Vagal modulation of the heart and central hemodynamics during dynamic handgrip exercise and forearm occlusion following forearm exercise training

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VAGAL MODULATION OF THE HEART AND CENTRAL HEMODYNAMICS DURING DYNAMIC HANDGRIP EXERCISE AND FOREARM OCCLUSION FOLLOWING FOREARM EXERCISE TRAINING

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ABSTRACT

The purpose was to examine the cardiovascular response to an acute bout of handgrip exercise before and after non-dominant arm exercise training. 19 people participated in 16 sessions of exercise training and 16 participants acted as controls (age: 20±1yrs). Blood flow measurements were taken at rest and following 3-min of forearm occlusion (RHBF) using plethysmography. Pneumotachometer, ECG, and blood pressure data were continuously collected during three testing conditions (spontaneous breathing (SB1: 5min), handgrip exercise (0.5hz) at 60%MVC with 50mmHg of pressure on the arm (H60+50mmHg: 5 min), and forearm occlusion (FAO: 3min)). Data were analyzed for respiratory rate, mean R-R interval, standard deviation of normal RR intervals (SDNN), normalized units of low- (0-0.15 hz) frequency power (LFnu), and mean arterial pressure (MAP). There was no main effect of group or arm. There was a main effect of test condition such that respiratory rate (+3.10±5.40breaths/min), LFnu (+19.06±14.73%), and MAP increased (+24.51±21.15mmHg) and mean R-R (-247.11±129.70msec) and SDNN (-45.16±40.65msec) decreased significantly during H60+50mmHg (non-dominant arm; $p<0.05$). Respiratory rate (-0.10±4.84breaths/min), SDNN (-9.50±57.14msec), and LFnu (-1.64±18.34%) recovered to SB1 levels during. Mean R-R (46.11±106.57msec) and MAP (16.65±15.84mmHg) remained elevated above SB1 ($p<0.05$). There were positive linear associations between forearm circumference and Mean R-R and MAP during H60+50mmHg; and MAP during FAO. There was a negative linear association with forearm circumference and Mean R-R during FAO. There was no significant main effect or interaction with handgrip exercise training on any of the variables. There was a

decrease in vascular resistance during RHBF (0.80±1.08 mmHg/ ml/100ml/min, $p<0.05$) in the arm that underwent exercise training. In conclusion, we found elevated MAP during FAO, which is indicative of significant EPR activity during exercise. Uniquely, we found linear associations between forearm circumference and the cardiovascular response to H60+50mmHg and FAO suggesting variation in the predominant mechanism of cardiovascular control. We did not see an attenuation of cardiovascular responses to H60+50mmHg and FAO with exercise training. However, we did see a decrease in forearm vascular resistance during the reactive hyperemia condition in the exercise-trained arm.

CHAPTER 1 INTRODUCTION

In response to the onset of exercise, heart rate and blood pressure rise to support working tissue. The initiation and magnitude of this response is a consequence of central and peripheral factors including higher brain center activity (anticipation of exercise) and mechanical compression and subsequent production of local metabolic by-products in the working musculature. Information from the higher brain centers and peripheral sites is received in various regulatory centers of the brain, which contribute to the modulation of various organs important in the exercise effort.

Arguably the most important regulatory center at the onset of the exercise effort is the cardiovascular control center located in the medulla oblongata. It is the cardiovascular control center, which enables rapid modulation of the cardiovascular system through parasympathetic withdrawal and increased sympathetic activation. The feedback loop between the local tissues involved in the exercise effort and the cardiovascular control center is known as the exercise pressor reflex. It is believed that receptors originating in the exercising skeletal muscle detect movement and the presence of metabolites released during exercise, and signal the vasoregulatory center of the brain through specific afferent tracks resulting in an efferent autonomic mediated responses (Rowell, 1997; Mitchell, Kaufman, Iwamoto, 1983; Thomas, Chavoshan, Sander, Victor, 1998).

The extent to which afferent information influences the autonomic response is poorly understood, but appears to have important clinical relevance. Recent literature suggests that dysfunction in skeletal muscle afferents may contribute to exercise intolerance in the elderly and those with heart failure (Piepoli, Ponikowski, Clark,

Banasiak, Capucci, Coats, 1999; Dinenno, Jones, Seals, Tanaka, 1999). Before we can understand how to intervene in clinically significant changes in EPR, we need to better understand EPR function during acute exercise in humans and the plasticity of EPR to exercise training or pharmacology. This may allow the development of treatment strategies for clinical populations effected by hypo- or hyperactive EPR.

There are a number of approaches that have been developed to identify the role of EPR during exercise. The EPR can be broken down into three distinct parts: the afferent arm of the reflex arc, the efferent arm of the reflex arc, and the end organ response to neural modulation via EPR.

Models to evaluate the role of EPR during exercise also can be categorized according to the part (described above) of the EPR from which they derive their information. The afferent models are considered the most highly controlled approach to evaluating the role of EPR during exercise, and directly measure afferent neural activity at the nerve or in the spinal cord. Models employing this technique have been generally performed on animals.

Alternately, direct nerve recordings from the efferent arm of the reflex arc can also be recorded and will be termed efferent models. Although experimental control over central command is less complete in these models it is possible to perform efferent measurement techniques in both animals and people, nor does such an approach require surgery or anesthesia.

The least invasive technique available to study EPR is the end organ approach. These techniques include measuring heart rate, heart rate variability, blood pressure, blood pressure variability, blood flow, and vascular resistance. Although this approach

has the lowest level of experimental control, it can be performed on animals and people without the complications of invasive techniques and provides integrated information about cardiovascular control.

Our lab has focused on using heart rate variability (HRV), an end-organ model, to measure autonomic modulation during exercise. Using an exercise protocol developed by Victor and Seals (1989) we found a dose dependent decrease in Mean R-R interval to dynamic handgrip exercise at 20%MVC and 60%MVC. However, MAP and low frequency normalized units (LFnu: an index of sympathetic modulation) increased only during 60%MVC handgrip exercise. These current data agreed with the results from previous research using other methods to measure autonomic modulation (Victor and Seals, 1989).

In addition to examination of the construct validity of this exercise protocol, we have also established the stability of cardiovascular responses to rest and exercise over a one-month period. Our data indicate that heart rate and indices of autonomic modulation are stable with R-values exceeding 0.72 at rest and during exercise (Kluess, Wood, Stone, Welsch, 2001). These values are consistent with previous reliability studies using heart rate variability to measure autonomic modulation of cardiovascular function (Amara and Wolfe, 1998; van de Borne, Montano, Zimmerman, Pagani, Somers, 1997). Therefore we have demonstrated an ability to measure cardiovascular responses to acute exercise.

The next step in this investigation was to test the specificity of the exercise protocol to the EPR. Post-exercise forearm occlusion is a commonly used test to determine the contribution of the EPR to the exercise performed. The theory is that if

EPR played a significant role in the muscle sympathetic and cardiovascular responses to exercise, then occluding the blood flow to the muscle after cessation of the exercise will result in a persistent elevation of muscle sympathetic activity and blood pressure due to continued stimulation of group IV afferents by the metabolites trapped in the muscle (Victor and Seals, 1989).

This effect has been demonstrated in both isometric and dynamic exercise using afferent, efferent, and end-organ models (McClosky and Mitchell, 1972; Seals, 1989; Victor and Seals, 1989; Hayward, Wesselmann, Rymer, 1991; Somers, Leo, Shields, Clary, Mark, 1992). While post-exercise forearm occlusion is a commonly used and accepted method to test for EPR activity during exercise, it is not without limitations. Using efferent or end organ models it is not possible to completely control, eliminate, or measure the influence of other cardiovascular control mechanisms that may also be acting on the system. However, using post-exercise forearm occlusion does allow us to make weak, but potentially important, inferences regarding the contribution of the EPR to the cardiovascular response to exercise.

Thus, we added post-exercise forearm occlusion to our current handgrip exercise model to evaluate the EPR specificity (Kluess, Wood, Stone, Wilson, Welsch, 2002). One problem we discovered with our current model is it did not result in statistically significant elevation of mean arterial pressure (MAP) above resting values in the control condition. While this is a common problem in small muscle mass activity it nevertheless, suggests that we did not achieve an optimal stimulation of group IV afferents during the exercise stimulus (dynamic handgrip exercise at 60%MVC) (Somers et al., 1992).

In contrast to our study, Victor and Seals (1989) found that dynamic handgrip exercise at 60%MVC was a sufficient exercise intensity to induce persistent elevation of sympathetic activity and blood pressure during post exercise forearm occlusion. One reason for this discrepancy between our study and Victor and Seals (1989) is that they used 2 minutes of exercise at 1 hz gripping frequency, while we used 5 minutes of exercise at 0.5 hz gripping frequency. Although the total work in our study and Victor and Seals (1989) were roughly matched, it appears that gripping cadence may play a larger role in the magnitude of sympathetic activity produced during exercise than previously understood.

In our original study, the participants had difficulty maintaining the 1 hz gripping frequency for five minutes. Previous work by this lab has demonstrated that it takes 60 to 90 seconds of handgrip exercise at 60% MVC (1 hz) to obtain a stable (steady state) heart rate (Kluess, Wood, Welsch, 2000). One of the assumptions of frequency domain analysis is that the signal is stationary, thus, a five-minute data collection time during exercise is critical to allow at least two minutes of data to be obtained in the steady state period (Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Therefore, we needed a change in the protocol that would increase sympathetic activity during dynamic handgrip exercise at 60%MVC (0.5 hz), but not reduce the duration of the activity.

Reducing metabolite washout by reducing the blood flow through the working muscle during exercise significantly increases the magnitude of sympathetic and cardiovascular response to a given exercise mode and intensity through increased

group III and IV afferents stimulation (Victor, Seals, Mark, 1987; Victor and Seals, 1989; Sinoway, Hill, Pickar, Kaufman, 1993; Adreani and Kaufman, 1998).

Primary evidence for using reduced metabolite washout during exercise to induce skeletal muscle afferents is a study by Adreani and Kaufman (1998) using a decerebrate cat model. They demonstrated that group III and IV afferents were directly stimulated with simulated walking activity with and without complete arterial occlusion. However, the magnitude of change in group III and IV afferent firing frequency to activity with arterial occlusion was 44% and 47% higher, respectively, than simulated walking without arterial occlusion.

In humans exercise with reduced metabolite washout has also been used to induce EPR activity. Mostoufi-Moab, Widmaier, Cornett, Gray, Sinoway (1998) used cardiovascular response to dynamic handgrip exercise at 25%MVC with 50 mmHg of pressure on the arm as a measure of EPR activity. A pressure of 50 mmHg on the arm has been well established as a pressure sufficient to occlude venous outflow, but have minimal influence on arterial inflow to the arm in humans (Cramer, Beach, Strandness, 1983). Therefore, exercise with 50 mmHg on the arm would cause metabolites to accumulate due to reduced venous outflow and stimulate the EPR.

Additionally, Victor et al. (1987) performed dynamic handgrip exercise at 30% MVC with 50mmHg of pressure on the arm and found that it resulted in approximately 3 fold greater heart rate and blood pressure response, compared to exercise with no pressure on the arm. They specifically attributed the cardiovascular and muscle sympathetic nerve activity responses to greater stimulation of the EPR through increased trapping of metabolites during the activity.

Therefore, reducing metabolite washout by applying 50 mmHg to the arm during exercise is an established model to induce EPR activity using both afferent, efferent, and end-organ models. In efferent and end-organ models, it is not possible to completely attribute measures of autonomic modulation and cardiovascular response to the EPR. However, when 50 mmHg of pressure is applied to the arm during exercise and is followed by forearm occlusion weak inferences can be made concerning the influence of the EPR during exercise.

Thus, we have modified our protocol to maximize skeletal muscle afferent contribution by adding inflation of a blood pressure cuff to 50-mmHg to the high intensity exercise. Occluding the arm during exercise has been shown to increase heart rate, blood pressure, and muscle sympathetic activity from 10 to 24% and should be a sufficient stimulus to optimally induce the EPR in the proposed study (Seals and Victor, 1991; Victor and Seals, 1989; Victor et al., 1987).

Subsequently, we wish to examine the effect of exercise training induced adaptations to EPR. To date, two studies have shown significant changes in cardiovascular responses to exercise with forearm occlusion and post-exercise forearm occlusion (Somers et al., 1992; Sinoway, Shenberger, Leaman, Zelis, Gray, Baily, Leuenberger, 1996; Mostoufi-Moab et al., 1998). However, all studies have used a training stimulus below that which is known to induce an increase in sympathetic drive from skeletal muscle afferents (less than 60%MVC)(Seals and Victor, 1991). Additionally, the need to evaluate the training effect with an EPR specific test is important.

Mostoufi-Moab et al. (1998) and Somers et al. (1992) did use appropriate tests for EPR, but Somers et al. (1992) was not able to induce a significantly elevated MAP during post-exercise forearm occlusion either before or after training. This suggests their exercise was not of a sufficient intensity to induce a large increase in sympathetic activity, previously established to be necessary to maintain group IV afferent activity during forearm occlusion (Victor and Seals, 1989).

Sinoway et al. (1996) did not test or train at an intensity believed to induce a significant increase in sympathetic activity. They concluded that the training adaptations from their protocol were due to mechanoreceptor adaptations, not metaboreceptor adaptations (Sinoway et al., 1996). Therefore, the ability of the EPR to adapt to exercise training is still not well understood and there is a need for a training and testing protocol specific to the EPR.

There is very little known concerning the chronic adaptations of the EPR. However, there is significant evidence that a change in autonomic modulation does occur with exercise training in as little as four weeks (Fisher and White, 1999; Schuit, van Amelsvoort, Verheij, Rijneke, Maan, Swenne, Schouten, 1999; Levy, Cerqueira, Harp, Johannessen, Abrass, Schwartz, Stratton, 1998; Seals and Chase, 1989; Somers et al., 1992; Mostoufi-Moab et al., 1998; Sinoway et al., 1996). Additionally, non-neural adaptations to exercise training such as vascular and skeletal muscle remodeling are known to be significant after four weeks of training and may indirectly influence EPR response to acute exercise and forearm occlusion (Sundberg, 1994 Mostoufi-Moab et al., 1998, Sinoway et al., 1996; Booth and Thomason, 1991; Laughlin, Oltman, and

Bowles, 1998). Therefore it is reasonable to assume that four weeks of training will be a sufficient and appropriate training period to see changes in cardiovascular response related to EPR activity.

Due to the non-specific nature of end-organ models to individual cardiovascular control mechanisms, it will not be possible to exclusively attribute training-induced changes in cardiovascular response to exercise and forearm occlusion to the EPR. However, using single-arm forearm training does provide an advantage over large muscle exercise in that the training effect is limited to the forearm and thus, changes in plasma volume, blood flow shunting, and other whole body adaptations will have a reduced effect on the cardiovascular changes observed (Fisher and White, 1999).

Before we can understand how to intervene in clinically significant changes in EPR, we need to know if EPR is modifiable through training and/or pharmacology. Improved understanding of the mechanism of change in autonomic modulation with training and the adaptability of EPR will allow development of treatment strategies for clinical populations effected by hyper- or hypo-active EPR.

Therefore, the purpose of this study was twofold: (1) to examine the cardiovascular response to an acute bout of handgrip exercise inasmuch as it may be reflective of stimulation of the EPR; and (2) to evaluate the influence of non-dominant handgrip exercise training on cardiovascular response to exercise and post-exercise forearm occlusion. We hypothesized that the modified exercise protocol would result in persistent elevation of MAP and the index of sympathetic activity (LFnu) during post-exercise forearm occlusion. Additionally, we hypothesized that exercise training will

result in attenuated HRV and blood pressure responses to exercise and recovery in the trained arm.

Previous studies have demonstrated that exercise training similar the current study, resulted in approximately 20% increase in maximal hyperemic blood flow following 5 minutes of forearm arterial occlusion (Alomari, Welsch, Prisby, Lee, Wood, 2001). Therefore, we hypothesized that blood flow would be higher in the trained arm following forearm occlusion compared to the control arm. Consistent with other studies, we also expected to see no significant changes in MVC and forearm circumference (Alomari et al., 2001; Somers et al., 1992; Mostoufi-Moab et al., 1998; Sinoway et al., 1996). Additionally, we expected no HRV, blood pressure, blood flow, MVC, or forearm circumference changes in the no treatment group.

1.1 Clinical Relevance

The extent to which afferent information influences the autonomic response is poorly understood, but appears to have important clinical relevance. Recent literature suggests that dysfunction in skeletal muscle afferents may contribute to exercise intolerance in the elderly and those with heart failure (Piepoli et al., 1999; Dinenno et al., 1999). Before we can understand how to intervene in clinically significant changes in EPR, we need to better understand EPR function during acute exercise in humans and the plasticity of EPR to exercise training or pharmacology. This may allow the development of treatment strategies for clinical populations effected by hypo- or hyperactive EPR.

Chronic changes in EPR sensitivity have been documented to occur as a result of aging and chronic heart failure. These changes in EPR are associated with both

decreased and increased EPR neural traffic and end organ response (Schuit et al, 1999; Dinenno et al., 1999; Zelis, Sinoway, Leuenberger, Clemson, Davis, 1991; Sverrisdottir, Rundqvist, Johannsson, Elam, 2000). Investigations concerning the role of EPR in exercise intolerance and cardiovascular response in aging and heart failure need further development. Establishment of exercise protocols as well as improved methods to study EPR contribution to autonomic modulation may aid in these studies. Additionally, the investigation of EPR as a contributor to exercise intolerance in other diseases such as peripheral vascular disease and diabetes should be advanced.

There is very little known concerning the chronic adaptations of the EPR. However, there is significant evidence that a change in autonomic modulation does occur with exercise training. Before we can understand how to intervene in clinically significant changes in EPR, we need to know if EPR is modifiable through training and/or pharmacology. Improved understanding of the mechanism of change in autonomic modulation with training and the adaptability of EPR will allow development of treatment strategies for clinical populations effected by hyperactive EPR.

1.2 Limitations

1. End-organ models provide only integrated information regarding cardiovascular control mechanisms. Therefore, it is not possible to completely attribute any change in cardiovascular response to exercise or forearm occlusion to the EPR.

2. Cardiovascular response to forearm occlusion may be confounded by factors such as baroreceptor sensitivity, pain response unassociated with group III and IV afferents, and/or changes in vascular function or control.

3. The training effect for the EPR may occur later than four weeks, therefore, we may see no change in cardiovascular response to exercise.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Cardiovascular control during exercise involves a complex system of redundant mechanisms that ensure adequate oxygen delivery to working muscles. These cardiovascular control mechanisms are both central and peripheral with each providing unique information to the brain.

The cardiovascular control center integrates information from all of the peripheral afferents and information from central command and the hypothalamus to create action through the autonomic branch of the nervous system. This branch of the nervous system has two distinct parts called parasympathetic and sympathetic. The parasympathetic branch of the autonomic nervous system originates in the nucleus vagi in the medulla and innervates the heart through the vagus nerve. The sympathetic branch of the autonomic nervous system originates in the cardiac accelerator nucleus of the medulla. The sympathetic branch innervates both the heart and the vasculature.

During exercise, the autonomic nervous system responds to reduce blood flow to non-working tissues and maximize blood flow to working tissues, while controlling blood pressure. This occurs initially through a withdrawal of parasympathetic nervous activity and an increase in sympathetic nervous activity, which result in increased heart rate, stroke volume, and vascular resistance in the GI tract and non-working tissues (Fagraeus and Linnarsson, 1976; Peterson, Armstrong, Laughlin, 1988; Rowell, 1997). The signals to induce autonomic nervous stimulation to various parts of the body during exercise are from central command, the hypothalamus, the respiratory stretch

receptors, O_2/CO_2 receptors, the arterial and cardiopulmonary baroreceptors, and skeletal muscle afferents.

Central command of cardiovascular control originates in the subthalamic locomotor region of the brain and acts to induce parasympathetic withdrawal and increase sympathetic activity by acting on the cardiovascular control center. The hypothalamus is active during exercise where there is an increase in body temperature. Then the hypothlalmus acts to vasodilate the skin blood vessels (Brooks, Fahey, White, 1996).

In the respiratory system there are several receptors that influence cardiovascular control. The respiratory stretch receptors are located in the chest wall and in muscles participating or assisting in respiration. When activated, their role is to inhibit respiration. Additionally, the respiratory system has receptors that detect O2, CO2, and pH. These receptors decrease or increase breathing rate through the medulla to optimize body levels of these elements (Brooks et al., 1996). In general, the arterial baroreceptors detect changes in arterial pressure and induce changes in autonomic nervous stimulation to maintain blood pressure within a narrow range. Since blood pressure increases during exercise, the baroreceptors are believed to reset to a new maintenance point. Therefore, during exercise the purpose of the baroreceptors is to maintain sympathetic activity and to prevent arterial hypotension (Rowell, 1997). The mechanism by which baroreceptor resetting occurs is not well understood, but may be influenced by skeletal muscle afferents which originate in the nucleus tractus solitarii as does the arterial baroreceptors reflex (Machado, Castania, Bonagamba, Salgado, 2000).

2.1.1 Skeletal Muscle Afferents

Cardiovascular control during exercise is also influenced by receptors that detect movement and metabolites in the muscle. Mechanoreceptors, such as golgi tendon organs and muscle spindles, detect movement and stretch within the tendons and muscle of active tissue. These receptors have no direct link to the cardiovascular control center, but do exert some influence through central command and possibly through interneuron links in the spinal cord with other skeletal muscle afferents (Thomas et al., 1998; Brooks et al., 1996; Appelberg, Hullinger, Johansson, Sojka, 1983; Pedersen, Ljubisavljevic, Bergenheim, Johansson, 1998).

One of the most interesting and controversial mechanisms of cardiovascular control originating in the skeletal muscle is the exercise pressor reflex (EPR). According to Rowell (1997), the purpose of these skeletal muscle afferents and their associated reflex is to "guard against hypoperfusion of the active muscle". This reflex arc begins with receptors in the skeletal muscle and interstitial spaces, which detect metabolites and movement in the working muscle. The information from the receptors is transmitted to the cardiovascular control center of the brain through afferent neurons called group III and group IV afferents. The action of this reflex is to increase sympathetic nervous stimulation to metabolically active muscular movement. Therefore, stimulation of the EPR results in increased heart rate, blood pressure, and vascular resistance.

Unlike mechanoreceptors, the EPR does have a direct connection with the cardiovascular control center of the brain. Evidence suggests that the ventrolateral medulla is a critical region for expression of the EPR. Li, Hand, Potts, Wilson, Mitchell

(1997), showed that the lateral reticular nucleus, the nucleus tractus solitarii, lateral tegmental field, the vestibular nucleus, the subretrofacial nucleus, and the A1 region of the medulla were activated with spinal stimulation to create static muscle contraction in anesthetized cats. Additionally, there is evidence that the area postrema in the dorsomedial portion of the medulla is involved in modulating the nucleus tractus solitarii. The nucleus tractus solitarii is also the primary baroreceptor reflex location, and therefore may be the source of interaction between the baroreceptor reflex and the EPR. Additionally, Bonigut, Bonham, Stebbins (1997) found that area postrema disruption resulted in enhanced blood pressure and heart rate response to static muscle contractions in anesthetized cats, suggesting that the area postrema plays an important role in baroreflex/EPR interaction. There is evidence that the baroreceptor reflex acts to buffer the hypertensive effect of the EPR (Raven, Potts, Shi, 1997).

The spinal cord is another source of EPR modification by peripheral mechanisms. The spinal cord portion of this reflex is a complex system of interneurons that may be modified by intraspinal bradykinin and/or glutamate (Stebbins and Bonigut, 1996; Adreani, Hill, Kaufman, 1996). This is believed, although not well studied, to be the source of mechanoreceptor/EPR interaction (Appelberg et al., 1983).

2.1.2 Morphology

Group III and IV afferents are a family of unencapsulated nerve endings connected to afferent nerves. Group III afferent endings are located in the interstitial space of the muscle, capillary walls, the venous vessel, the adventitia of lymph vessels, in connective tissue of the peritoneum, and in the collagen fiber bundles of the tendon (Andres, von During, Schmidt, 1985; MacLean, LaNoue, Gray, Sinoway, 1998). The

afferent nerve of group III afferents are small (1-4micrometers), thinly myelinated nerve fibers with conduction velocities of 2.6 to 30 meters/second (Rotto and Kaufman, 1988; Thomas et al., 1998; Paintal, 1960). Group IV afferents nerves are small (1-4micrometers), unmyelinated nerves with conduction velocities less than 2.5 meters/second (Andres et al., 1985; Rotto and Kaufman, 1988). Group IV afferent endings contain granulated vesicles and most likely release protein neurotransmitters like substance P. They are located extensively in the blood vessels, lymphatic vessels, and the interstitial space in the muscle (Andres et al., 1985; MacLean et al., 1998). Although we know a great deal about the distribution and basic anatomy of GIII and GIV afferents, the mechanism by which GIII and GIV afferents detect metabolites is, to date, undiscovered. However, there is evidence that GIII and GIV afferents respond to metabolites such as lactate, adenosine, leukotrienes, prostaglandins, hydrogen ions, and potassium ions released by the muscle (Thomas et al., 1998).

2.2 Models That Have Been Used to Evaluate EPR

Despite more than 70 years of study a great deal of controversy still exists concerning the exercise pressor reflex (Mitchell et al, 1983). From this controversy a number of hypotheses have arisen. One set of hypotheses is the central command versus the exercise pressor reflex theory of cardiovascular control during exercise. The Central command theory states that higher brain centers direct all cardiovascular control and motor unit recruitment during exercise, while the exercise pressor reflex theory states that peripheral mechanisms play an important role in cardiovascular control (Mitchell et al., 1983). To date, there is good evidence that central command in combination with peripheral afferents influence cardiovascular control during exercise.

However, the set of stimuli that influences the predominance of one mechanism of cardiovascular control versus another is still under debate. Within EPR literature, the sets of hypotheses are called the traditional versus the emerging hypothesis of EPR function. The traditional hypothesis states that EPR only influences cardiovascular control under conditions where there is a mismatch between blood flow and skeletal muscle demand. The emerging hypothesis states that EPR is active and influencing cardiovascular control both during low intensity exercise and under conditions where blood flow and demand are not matched (Thomas et al., 1998). Past and current research has provided some insight into the validity of these hypotheses, but a great may questions still need answering to further understand the mechanisms of cardiovascular control during exercise.

There are a number of approaches that have been developed to identify the role of EPR during exercise and test the exercise pressor reflex theory and the emerging model of metabolite-sensitive afferents in acute exercise. The EPR can be broken down into three distinct parts: the afferent arm of the reflex arc, the efferent arm of the reflex arc, and the end organ response to neural modulation via EPR.

Models to evaluate the role of EPR during exercise also can be categorized according to the part (described above) of the EPR from which they derive their information. The afferent models are considered the most highly controlled approach to evaluating the role of EPR during exercise, and directly measure afferent neural activity at the nerve or in the spinal cord. Models employing this technique have been generally performed on animals and tend to focus on central command versus EPR theory of cardiovascular responses to exercise.

Alternately, direct nerve recordings from the efferent arm of the reflex arc can also be recorded and will be termed efferent models. Although experimental control over central command is less complete in these models it is possible to perform efferent measurement techniques in both animals and people, nor does such an approach require surgery or anesthesia.

The least invasive technique available to study EPR is the end organ approach. These techniques include measuring heart rate, heart rate variability, blood pressure, blood pressure variability, blood flow, and vascular resistance. Although this approach has the lowest level of experimental control, it can be performed on animals and people without the complications of invasive techniques and provides integrated information about EPR. While each of these models has limitations, all have provided unique and valuable information regarding the role of EPR under various circumstances and the conditions under which EPR functions.

2.2.1 Afferent Models

The most powerful model providing information regarding the role of EPR in cardiovascular control during exercise is the decerebrate cat model. In this model, the cerebrum of the cat is removed, thus eliminating central command by higher brain centers but allowing breathing, heart rate, and neural transmission to continue without the interference of anesthesia. Exercise activity is produced at the spinal cord through stimulation of the ventral nerve roots. This model has provided valuable evidence supporting the existence of the EPR and highlighting some of the characteristics of EPR.

The first study using this model was McMahon and McWilliams (1992). Using electrical stimulation of the sciatic nerve (10 and 50 hz pulse of 1 ms duration - contract lasted 5 sec), McMahon and McWilliams (1992) found the latency of cardiac acceleration following muscle contraction was 687 ms and suggesting that early changes in R-R interval can be controlled from reflex activity in the working muscles. Additionally, a further increase in reflex R-R interval response was eliminated by a bolus of atropine indicating cardiac acceleration in response to muscular activity was due to vagal withdrawal.

The characteristics of EPR were further explored by MacLean et al. (1998). They used 3 and 5 hz twitch contractions of the sciatic nerve for 0.1 ms duration for 5 minutes. Additionally, they included micro dialysis probes in the muscle to measure intramuscular concentrations of metabolites to evaluate the most likely metabolite that stimulates EPR. They found a dose dependent heart rate response, but MAP only increased at the highest stimulation rate. Lactate, phosphate, hydrogen ions, and potassium ions were all elevated during both stimulation frequencies, however, following 10 minutes of recovery, lactate and hydrogen ion concentrations were still elevated even though heart rate and blood pressure had returned to normal. Thus, they concluded that EPR is also stimulated during lower intensity longer duration activity and that potassium is the most likely metabolite stimulus for EPR activity.

Another study by Adreani, Hill, Kaufman. (1997) showed that simulated walking activity in decerebrate cats directly stimulated the group III and IV afferents measured at the spinal cord. A further study by Adreani and Kaufman (1998) demonstrated that group III and IV afferents were also directly stimulated with simulated walking activity

during arterial occlusion. However, the magnitude of group III and IV afferent response to ischemic activity was 44% and 47% higher, respectively, than simulated walking without arterial occlusion. Interestingly the hydrogen ion and lactate ion concentration in the venous blood was not significantly different in the ischemic and non-ischemic states, thus, suggesting that a metabolite other than H+ and lactate are responsible for EPR during ischemia.

In summary, the decerebrate cat model has established that, in the absence of higher brain centers, a reflex originating in the muscle increases heart rate and blood pressure by initially stimulating vagal withdrawal. This reflex operates at maximal isometric workloads, very low intensity dynamic activities, and under conditions of impaired blood flow which support the emerging model of EPR activity. The response of this reflex appears to be dose dependent, but the metabolite that stimulates group III and IV fibers is still under investigation.

Another method by which central control is reduced is the anesthetized animal model. Although this model is less technically rigorous than the decerebrate cat model, the presence of anesthesia during testing may alter autonomic function and nerve impulses and thus, may change the behavior of the reflex or the actions resulting from an active EPR. Despite the problems with this model, it has been used to provide important information concerning the characteristics of EPR under conditions of reduced input from central command.

Rotto and Kaufman (1988) used this model to investigate the metabolic by-product stimulating EPR activity. They injected the femoral artery of anesthetized cats with lactic acid, arachidonic acid, adenosine, and phosphate at rest. They found that

infusion of lactic acid (400mM) resulted in increased the discharge rate of 69% of the GIII and GIV afferents (latency time 5-10 sec) studied and caused an increase in arterial pressure in 22 out of 30 trials. Arachidonic acid (AA) injection resulted in a 50% increase in discharge frequency (latency of 1-3 min) that lasted for 2-5 min after the injection. The addition of indomethacin attenuated the response to AA by 83%. Interestingly, AA caused a decrease in arterial pressure of 10 to 76 mmHg in 33 out of 59 trials. Adenosine and phosphate caused 17% or fewer of the afferents studied to increase their discharge frequency at rest. Therefore Rotto and Kaufman (1988) concluded that lactate and cyclooxygenase products are the most likely metabolites responsible for EPR.

One of the more unique studies using this model was Sinoway et al., (1993). They found that group III discharge frequency increased with the injection of La at rest and decreased when dichloracetate (DCA), a drug that inhibits lactate dehydrogenase, was added. However, repeated exposure to lactate at rest reduced the discharge frequency. This reduction in discharge frequency was reversed by muscle contraction. Thus, suggesting that the metabolite receptors (group III afferents) have a relationship with receptors of movement (group IV afferents).

In summary, this model has been primarily used to determine the metabolite(s) responsible for stimulating the EPR under conditions of reduced influence from higher brain centers. Although still controversial, the results of these studies suggest that the most likely metabolites that stimulate EPR during isometric and dynamic exercise are lactate and cyclooxygenase products. This conclusion is in conflict with the findings of MacLean et al. (1998) who employed a decerebrate cat model and inter-muscular

microdialysis. While microdialysis metabolite collection is a superior method of determining physiological levels of metabolites that may effect EPR, MacLean et al., (1998) based their elimination of lactate as an important EPR activating metabolite on the evidence that HR and BP recovered from exercise before the lactate concentrations. Although higher brain centers were eliminated in their experiment, they failed to consider other autonomic reflexes that may play a predominant role during recovery from exercise, such as the baroreceptor reflex. These findings do not conflict with the findings of Adreani and Kaufman (1998) that suggested that lactate may not be the predominant metabolite responsible for EPR activity during ischemia. Both of these models suggest strongly that there is a continuum of group III and IV afferents that respond to a variety of combinations of metabolites, muscular activity, and blood flow.

2.2.2 Efferent Models

Models like the decerebrate cat and anesthetized animal strongly support the existence of the exercise pressor reflex and have demonstrated that when central command is eliminated or reduced, this mechanism stimulates heart rate and blood pressure to increase. These models also strongly support the emerging model of afferent contribution to cardiovascular control in that GIII and GIV afferents increased discharge frequency to low intensity electrical stimulation as well as maximal tetanic stimulation and ischemia. However, these models have provided no evidence that this reflex plays a role during conscious activity and that the EPR occurs in humans to the same degree as cats and rabbits.

Efferent models have become common to evaluate the role of EPR during various exercise protocols with recent improvements in the technique of

microneurography. Microneurography involves inserting a tungsten electrode into a nerve near the skin surface. The electrode is moved until a signal consistent with sympathetic efferent activity is detected (Seals and Victor, 1991). These data are commonly expressed as either the number of sympathetic bursts per minute or as sympathetic bursts per minute multiplied by the mean burst amplitude of the condition and in termed muscle sympathetic nerve activity (MSNA).

The advantage of efferent models is that, although they are invasive, they do not require anesthesia. Another advantage is that they measure peripheral sympathetic neural output as a result of reflex activity. A disadvantage is these protocols cannot eliminate the contribution of central command to the sympathetic output measured and therefore cannot infer with a great deal of certainty that GIII and GIV afferent activity caused the sympathetic response measured in these experiments. However, an indirect approach for indicating GIII and GIV involvement used in efferent models is measuring peripheral sympathetic output during post-exercise ischemia. This procedure theoretically prevents metabolites created during the previous exercise protocol from leaving the area and thus, continues to act on the GIII and GIV receptors. Therefore, the result of this manipulation is that sympathetic outflow and, subsequently, BP remains elevated above rest, allowing the suggestion that the exercise responses were due to EPR activity (Seals and Victor, 1991).

Despite the problems associated with efferent models, the experiments performed have provided valuable information regarding human sympathetic responses to various exercise protocols, active muscle masses, and magnitude of blood flow

restriction. The most common exercise mode for use with microneurography recording is isometric exercise of the forearm, typically lasting from 3 to 5 minutes.

Figure 2.1 summarizes the current literature regarding MSNA responses in bursts per minute to isometric exercise at varying exercise intensities (symbol F). Each point represents the peak mean change from resting MSNA (range: 15 to 38 bursts/min) for each study. Isometric handgrip exercise results in a significant increase in the sympathetic activity going to the forearm even at exercise intensities as low as 10% of the participant's maximal voluntary contraction (MVC) in healthy adults (Saito, Iwatse, Mano, 1986; Seals, 1989). This dose dependent effect was also seen with significant mean changes in heart rate and blood pressure from rest. However, blood pressure did not increase significantly until MSNA increased to 6 bursts per minute.

Dynamic exercise has a much different pattern of nervous and cardiovascular response to exercise compared to isometric exercise. The results from a number of studies using dynamic exercise intensities of 10% MVC to 60% MVC and a gripping frequency of 0.7 to 1 hz are summarized in figure 2.1 (symbol ∇). Dynamic handgrip activity does not result in a significant increase in MSNA until 60% MVC. However, the change in heart rate and blood pressure from rest occurs with dynamic handgrip exercise at 10%MVC (Victor et al., 1987; Victor and Seals, 1989; Saito et al., 1986). When larger muscle groups are used, such as with arm cycling (symbol □ in figure 2.1), MSNA significantly increases from rest (25 burst/min difference) at 30% of MVC and is accompanied by heart rate (32 beats/min difference) and blood pressure (25 mmHg difference) changes (Victor et al., 1987; Victor and Seals, 1989).

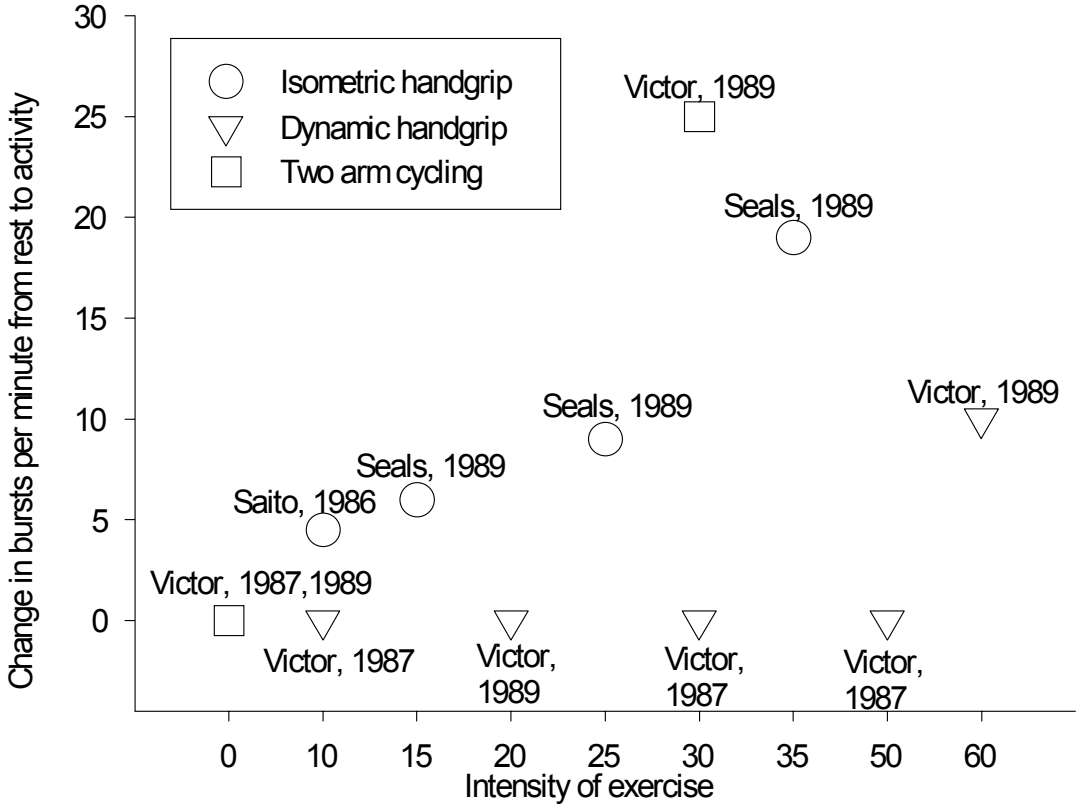

Figure 2.1: Summary of MSNA results in bursts per minute for microneurography literature over different exercise protocols and exercise intensities.

Therefore, the sympathetic response to isometric handgrip activity appears to have a lower threshold for increasing sympathetic activity and cardiovascular response than dynamic handgrip activity. However, the magnitude of cardiovascular response at 10 bursts/min above rest is greater with dynamic, than isometric handgrip exercise.

Additionally, an increase in sympathetic activity during dynamic activity in larger muscle masses occurs at a lower relative workload than smaller muscle masses, but induces a larger magnitude change in sympathetic and cardiovascular response. Seals (1993), comparing isometric handgrip exercise at 30% MVC and isometric abduction of the FDI muscle of the hand, further illustrated the muscle mass effect. They found that both the magnitude of increase in sympathetic activity and cardiovascular response was 2 to 3-fold greater in the larger muscle mass compared to the smaller muscle mass.

Reducing blood flow to the working muscle and following exercise also significantly increases the magnitude of sympathetic and cardiovascular response to a given exercise mode and intensity. Victor et al., (1987) performed dynamic handgrip exercise at 30% MVC both with and without blood flow restriction. Exercise with blood flow restriction resulted in approximately 3 fold greater heart rate and blood pressure response, compared to exercise with no restriction. Additionally, dynamic handgrip exercise at 30%MVC with normal blood flow did not result in an increase in sympathetic outflow, but when blood flow was restricted during exercise MSNA was 16 bursts/min higher than rest. This magnitude of change in sympathetic activity is similar to the magnitude of change seen with 35% of isometric activity (19 bursts/min above rest (Seals, 1989).

Interestingly, the dose dependent effect described above does not occur with larger muscle masses. Arm cycling with no resistance and blood flow restriction (250mmHg) resulted in a 2.7-fold greater heart rate, a 2-fold greater blood pressure response, and a sympathetic increase of 25-bursts/min above rest (similar to arm cycling at 30% normal blood flow). However, arm cycling at 30% of the maximal workload with restricted blood flow resulted in only a 1.3-fold increase in HR, 1.6-fold increase in BP, and a 2.4-fold increase in MSNA, compared to the same exercise load with no blood flow restriction (Victor and Seals, 1989). This protocol was also performed with 40 mmHg and 100 mmHg on the cuff during no load and 30% of the maximal load arm cycling activity. These pressures did not result in a change in the sympathetic or cardiovascular response to the activity.

Post-exercise forearm occlusion is a commonly used test to determine the contribution of the EPR to the exercise performed. The theory is that if EPR played a significant role in the muscle sympathetic and cardiovascular responses to exercise, then occluding the blood flow to the muscle after cessation of the exercise will result in a persistent elevation of muscle sympathetic activity and blood pressure due to continued stimulation of group IV afferents by the metabolites trapped in the muscle (Victor and Seals, 1989).

Seals (1989) had their participants perform isometric handgrip exercise at 35%MVC and then post-exercise occlusion. They found a persistent elevation of muscle sympathetic activity and calf vascular resistance above resting values. This effect did not occur when participants were allowed to recover without blood flow restriction. In agreement with these findings, Somers et al. (1992) found a 270% increase in MSNA from rest during forearm occlusion following 33% isometric handgrip exercise. However, heart rate and blood pressure were not significantly different from rest. Following dynamic handgrip activity at 60%MVC, Victor and Seals (1989) found a persistent elevation of MSNA and blood pressure, but not heart rate with post exercise occlusion. However, post exercise occlusion had no effect on neural or cardiovascular responses to dynamic handgrip exercise at 20%MVC.

Data from both dynamic handgrip and arm cycling suggest that restricting blood flow lowers the threshold for increasing sympathetic activity and increases the magnitude of the cardiovascular response for a given intensity of activity. MSNA appears to plateau in two arm cycling when blood flow is restricted regardless of the resistance on the flywheel. These results from arm cycling activity suggest that there is

an upper limit for increasing sympathetic activity in upper extremity activity. Additionally, post exercise occlusion appears to maintain exercise-induced increases in muscle sympathetic activity following both isometric and dynamic handgrip exercises. However, when the exercise did not result in a significant increase in sympathetic outflow, post exercise occlusion had no effect on neural or cardiovascular responses during recovery.

2.2.3 End Organ Models

The terminal end of any reflex loop is the target or end organ. In the exercise pressor reflex, the target organs are the heart and the vasculature. End organ models for EPR include measurements of heart rate, blood pressure, heart rate variability, blood pressure variability, blood flow, vascular resistance, and vascular conductance. Measuring the response of these organs has provided important information regarding autonomic control at rest and during exercise. End-organ models are the least specific models to measure EPR influence over cardiovascular control, therefore, attributing the end organ response exclusively to the EPR is difficult.

An advantage of these models is there are no mode, intensity, or muscle group restrictions for exercise in animals and people. Additionally, end organ models are generally non-invasive, therefore, making it less likely that measurement itself is altering normal response and does not require anesthesia. An added advantage of end organ models is both parasympathetic and sympathetic information can be inferred, allowing a more complete view of autonomic control of the cardiovascular system during exercise.

Measurements of heart rate and blood pressure alone give us only very general information regarding autonomic modulation at rest and during exercise. However,

analyzing heart period variation provides a unique measurement of autonomic modulation of the cardiovascular system.

The theoretical framework for heart rate variability (HRV) as a measure of autonomic modulation is based on the cardiac tissue processing of signals from the two branches of the autonomic nervous system. The parasympathetic branch of the autonomic nervous system releases acetylcholine at the post ganglionic synapse which acts directly on the SA node and atrial conduction fibers to open potassium channels and hyperpolarize the cells. Acetylcholine is then rapidly broken down by acetylcholinesterase, which stops the hyperpolarization stimulus. Therefore, parasympathetic neural activity has a very rapid tissue response to stimulation and a rapid recovery from stimulation, thus exerting its hyperpolarizing influence at a high frequency (0.15hz to 0.40 hz) (Task Force, 1996; Guyton and Hall, 2000).

In contrast, the postganglonic fibers of the sympathetic nervous system release norepinephrine at the synapse, which stimulates beta-receptors to initiate the second messenger cascade for cAMP. This eventually results in opening of calcium and sodium channels in the tissue and results in tissue excitability. Sodium and calcium pumps removing the ions from the target cells cause recovery from sympathetic neural activation. Therefore, sympathetic neural activity has a very slow onset of tissue response and a slow recovery from stimulation resulting in a low frequency of neural activity (0.04 hz to 0.15 hz)(Task force, 1996; Guyton and Hall, 2000).

Predominant parasympathetic control of the heart results in high variation in the R-R interval due to the rapid onset and recovery of the cardiac tissue to parasympathetic nervous activity. Sympathetic stimulation of the heart results in a

higher heart rate with less variability because of the prolonged tissue excitability induced by sympathetic neural input. Through this hypothesis it is possible, in general, to suggest that the standard deviation of the normal sinus R-R intervals (SDNN) indicate a predominant parasympathetic or sympathetic modulation of the heart rate (Task force, 1996).

Evidence to support SDNN as a measure of autonomic modulation is that when in animals or humans the Vagus nerve is cut, the SDNN decreases (Jokkel, Bonyhay, Kollai, 1995; Task force, 1996). Additionally, during exercise, when parasympathetic withdrawal and increase in sympathetic stimulation are known to occur, SDNN decreases (Kluess et al., 2000; Iellamo, Pizzinelli, Massaro, Raimondi, Peruzzi, Legramante, 1999). Studies using paced breathing to induce an increase in parasympathetic modulation of the heart have found an increased SDNN (Cooke, Cox, Dietrich, Taylor, Beightol, Ames, et al., 1998; Lee, Aucoin, Wood, Welsch, 2000; Wood, LeLeux, Welsch, Nelson, Kluess, Lee, 2001). SDNN also has predictive validity.

There have been a number of studies in adults and infants to suggest that lower SDNN taken over a 24-hour period predict an increased risk for sudden death (Lombardi, 2000). However, SDNN does not discriminate between the modulation by branches of the autonomic nervous system and is influenced by the heart rate at which the measurement was taken, thus reducing the value of comparison between people and during exercise (Kluess et al., 2000).

Another measure of heart rate variability is the frequency domain. This technique is based on the previously mentioned frequency differences between the branches of the ANS. Using frequency analysis, this technique attempts to discriminate

between R-R intervals occurring in a frequency band of parasympathetic neural stimulation of the heart (high frequency: 0.15 hz to 0.40 hz) and in a frequency band of sympathetic neural stimulation of the heart (low frequency: 0.04 hz to 0.15 hz) (Task force, 1996).

The validity of assigning various frequencies to parasympathetic and sympathetic modulation is based on a number of studies. Frequency distributions of the R-R intervals from people with heart transplants and animals after undergoing surgical vagotomy and sympathectomy have shown no power in the frequency bands assigned to ANS neural input (Task force, 1996). In neurally intact animals and humans, beta blockade resulted in a reduction in the low frequency power.

Using the parasympathetic blocker atropine reduced the power of the high frequency domain, but also decreased the power in the low frequency domain somewhat (Task force, 1996). Exercise causes a reduction in the high frequency power, and an increase in the low frequency power, but also causes a reduction in the total power of the frequency spectrum (Iellamo et al., 1999; Gonzalez-Camarena, Carrasco-Sosa, Roman-Ramos, Gaitan-Gonzalez, Medina-Banuelos, Azpiroz-Leehan, 2000).

2.2.3.1 HRV as a Measurement of Autonomic Modulation

Few studies to date have used to HRV as a measurement of exercise induced change autonomic modulation. Fagraeus and Linnarsson (1976) reported that initial changes in heart rate during exercise were primarily due to parasympathetic withdrawal. Additionally Hollander and Bouman (1975) demonstrated, using pharmacological

blockade, that the decrease in R-R interval during low intensity exercise was mediated by vagus nerve activity without an increase in sympathetic drive.

More recent work by Kurita, Takase, Hikita, Uehata, Nishioka, Nagayoshi, et al. (1999) found that heart rate, blood pressure, and high frequency power increased in response to isometric handgrip exercise, but low frequency power and cardiac norepinephrine did not change from rest to exercise. Iellamo, et al. (1999) used 4 minutes of static leg extension at 30%MVC followed by 5 minutes of post exercise leg occlusion. They found normalized low frequency power increased with static leg exercise and remained high during post exercise occlusion. High frequency normalized power decreased during exercise, but returned to baseline levels during post exercise occlusion. They concluded that EPR was active during exercise and post exercise occlusion. Heart rate was predominantly controlled by the baroreceptor reflex during post exercise occlusion, but blood pressure remained elevated due to EPR.

Gonzalez-Camarena et al. (2000) compared the HRV response of isometric (30%MVC) and dynamic (30 to 60%VO_2max, 60 rpm, 6 min) quadriceps femoris exercise. Isometric exercise resulted in higher SDNN and heart rate compared to rest and both dynamic exercise intensities. Low frequency and high frequency power was significantly higher during isometric exercise compared to rest and both dynamic exercise intensities. The HF normalized units were higher with isometric exercise, compared to both dynamic exercise intensities. They concluded that the cardiovascular responses to isometric and dynamic exercise are not controlled through the same mechanisms and speculated that the baroreceptor reflex may play a more dominant role in isometric compared to dynamic exercise.

Additionally, our lab has established the validity and reliability of using HRV as a measure of autonomic modulation during dynamic handgrip activity (Kluess et al., 2000; Kluess et al. 2001). We found a dose dependent response of heart rate, frequency domain indices of autonomic modulation and blood pressure during low (20%MVC) and high (60%MVC) intensity dynamic handgrip exercise. SDNN did decrease with exercise, but was not dose dependent. These data agreed with the results from Victor and Seals (1989) using MSNA as a measure of sympathetic modulation. Thus, we were successful in observing changes in autonomic nervous system modulation and that autonomic change was accompanied by dose dependent changes in heart rate and blood pressure.

In addition to testing the constructive validity of this protocol, we have also established the stability of cardiovascular responses to rest and exercise over a one-month period. Our data indicated that heart rate and indices of autonomic modulation are stable with R-values exceeding 0.72 at rest and during exercise (Kluess et al., 2001). These values are consistent with previous reliability studies using heart rate variability to measure autonomic modulation of cardiovascular function.

Therefore, HRV can be used to assess autonomic modulation of the heart during rest and exercise with results that agree with efferent models. Iellamo, et al. (1999) demonstrated that HRV can be used to assess autonomic modulation during post exercise occlusion, and therefore, may be a suitable non-invasive tool to better understand the role of the EPR during exercise.

2.2.4 Blocking the EPR

One of the problems with both efferent and end-organ models are the lack of specificity to a particular mechanism of cardiovascular control. One method to overcome this problem is using a chemical that block the mechanism of cardiovascular control you wish to study. A disadvantage of using a blocker is, by altering the response of one of the mechanisms of cardiovascular control, it may alter the behavior of other mechanisms. Despite this disadvantage, blockers have been used to assess the role of EPR in cardiovascular control during exercise.

Blockers that have been used in EPR research focus on reducing the amount of metabolite production during exercise. Therefore, cyclooxygenase inhibitors, such as aspirin, ketoprofen, and indomethicin, which block prostaglandin production, have been successful in reducing the cardiovascular responses to exercise. Additionally, dichloracetate and glycogen depletion, which reduces lactate production also, reduces EPR response.

Rotto et al. (1990) found that Aspirin and indomethacin attenuated group IV afferent discharge by ~82% during a two stimulated muscle contractions in anesthetized cats. Darques, Decherchi, and Jammes (1998) using aspirin and dicloracetate in paralyzed and anesthetized rabbits during exercise supported these findings. They found a 44 to 75% decreased group III and IV afferent activity with the blockers during muscle stimulation with no change in resting afferent activity.

The effect of ketoprofen (1 mg/kg of body weight) in humans during static handgrip activity has also been studied. Fontana et al. (1994) found a 55% decrease in respiratory frequency, 22% decrease in mean arterial pressure, and an 11% decrease

in heart rate. EPR in humans also appears to be lactate sensitive. Sinoway, Wroblewski, Prophet, Ettinger, Gray, Whisler, et al. (1992) found a 12% decrease in blood pressure (BP) during post-leg exercise occlusion following glycogen depletion to reduce lactate production.

Our lab has also attempted to develop a blockade model for EPR. We used Aspirin (ASA) at doses of 80 mg, 325 mg, and 650 mg during low intensity dynamic handgrip exercise (20%MVC), high intensity dynamic handgrip exercise (60%MVC), forearm occlusion following 60%MVC exercise, and rest. We found lower indices of sympathetic activity during post exercise forearm occlusion with 80 mg ASA; higher systolic blood pressure during post exercise forearm occlusion with 325 mg ASA; but higher spontaneous breathing R-R interval with 650 mg ASA compared to the control visit (Kluess et al., 2002).

Our conclusions were that reduction in sympathetic activity during forearm occlusion with 80-mg of ASA was most likely a result of attenuated group IV afferent firing. Additionally, ingestion of 325-mg of ASA resulted in increased systolic blood pressure during forearm occlusion and is consistent with changes in vascular compliance. This effect was reversed with 650-mg of ASA and resulted in lower resting heart rate most likely a result of a change in intrinsic heart rate, rather than a change in autonomic modulation. These findings suggest that aspirin influences cardiovascular control even during small muscle activity and suggest that aspirin in doses of 325-mg or more has a broad spectrum influence on the body (Kluess et al., 2002).

Therefore, chemicals that block the production of prostaglandins, leukotrienes, and lactate result in reduced group III and IV afferent firing in cats and rabbits. In

humans, cardiorespiratory response to arm and leg activity is significantly reduced when chemicals that block the cyclooxygenase pathway or lactate production are used.

2.3 Chronic Changes in EPR Activity

While there is good evidence that the EPR exists, and responds to metabolic and movement stimuli, there is little evidence concerning EPR adaptations to exercise training. However, there is evidence that autonomic nervous system modulation changes as a result of exercise training in younger people and good evidence that exercise training alters autonomic modulation in older people, people with heart disease, and people with renal disease, but the mechanism of these changes are poorly understood (Levy et al., 1998; de Meersman, 1993; Ng, Callister, Johnson, Seals, 1994; Braith, Welsch, Feigenbaum, Kluess, Pepine, 1999; Deligiannis, Kouidi, Tourkantonis, 1999; Duru, Candinas, Dziekan, Goebbels, Myers, Dubach, 2000; Stein, Ehsani, Domitrovich, Kleiger, Rottman, 1999; Hull, Vanoli, Adamson, Verrier, Foreman, Schwartz, 1994).

2.3.1 Forearm Exercise Training

Forearm exercise training is the most common mode for attempting to separate out various mechanisms of cardiovascular control because it allows some separation between changes in central command and peripheral mechanisms of cardiovascular control (Fisher and White, 1999). Additionally, with forearm training, the training effect is limited to the forearm and thus, changes in plasma volume, blood flow shunting, and other whole body adaptations have a reduced effect on the cardiovascular changes observed.

Somers et al. (1992) had 8 men perform 6 weeks of dominant arm training at 30%MVC. They found no difference in heart rate or MAP response to isometric handgrip exercise at 33%MVC, but MSNA response was significantly lower during exercise and post exercise forearm occlusion following training. Mostoufi-Moab et al. (1998) trained the non- dominant arm in 10 men, 5 days per week for 4 weeks at 30%MVC. They found that training had no effect on cardiovascular and metabolic response to rhythmic handgrip activity at 25%MVC. However, when this exercise was performed with 50 mmHg of pressure on the arm, the trained arm MAP ( )12 mmHg), lactate, and pH response to activity was decreased. They concluded that the EPR was modified as a result of training.

Sinoway et al. (1996) used the same training protocol as Mostoufi-Moab et al. (1998) and found an increased MSNA and forearm resistance and a decreased change from rest in lactate, MAP, arterial norepinephrine, and forearm blood flow during rhythmic handgrip exercise at 25%MVC. Since they saw these changes at an exercise intensity that was below that which is believed to activate group IV afferents, they concluded that this training changes mechanoreceptors (group III afferents), but not the metaboreceptors (group IV afferents).

One of the potential reasons that there are mixed results regarding forearm training and EPR is the intensity of the training itself. All three studies mentioned above used a training intensity below that which is believed to induce an increase in sympathetic activity caused by EPR (Seals and Victor, 1991). Additionally, the need to evaluate the training effect with an EPR specific test is important. Mostoufi-Moab et al. (1998) and Somers et al. (1992) did use appropriate tests for EPR, but Somers et al.

(1992) was not able to induce a significantly elevated MAP during post-exercise forearm occlusion either before or after training. This suggests their exercise was not of a sufficient intensity to induce a large increase in sympathetic activity, previously established to be necessary to maintain group IV afferent activity during forearm occlusion (Victor and Seals, 1989).

Sinoway et al. (1996) did not test or train at an intensity believed to induce a significant increase in sympathetic activity. They did see blood pressure and blood flow changes at 25%MVC which has not been previously shown. However, Sinoway, et al. (1996) used an intra-arterial pressure gauge to measure BP. The higher sensitivity of this instrument as compared to auscultory BP measurements explains this result.

2.3.2 Autonomic Modulation Following Exercise Training

While forearm exercise training is the most specific method for identifying mechanisms of cardiovascular control changes with exercise training, there is little evidence to support EPR-specific changes in autonomic modulation. However, there is good evidence that large muscle exercise does result in a change in autonomic modulation, both at rest and during exercise.

Fisher and White (1999) used dynamic heel raise exercise training for 6 weeks and found a 28% reduction in diastolic blood pressure and heart rate to isometric exercise at 30% MVC, with no change in cardiovascular response to post exercise leg occlusion compared to pre-training. However, they also observed a 24% decrease in diastolic pressure and heart rate in the untrained limb. They concluded that the training resulted in adaptations to central command.

Schuit et al. (1999) had healthy people between the ages of 60 and 80 years aerobically train for 6 months. They found a 6 to 15% increase in 24-hour HRV parameters following training. Additionally, Stein et al. (1999) found a 13% increase in SDNN and a 30% increase in VO_2max following 9 months of moderate intensity exercise training in people in their 60's.

Short term HRV (collection time of 5 minutes or less) has not been used frequently for evaluating the training effect. Levy et al. (1998) aerobically trained younger (range: 24-32 years) and older men (range: 60-82 years) for 6 months. HRV was collected in two-minute intervals at rest and during each stage of a cycle ergometer graded exercise test. They found a higher standard deviation of the normal R-R intervals (SDNN) in both groups and no significant difference between the groups following training. The decrease in SDNN from baseline to 400 kpm was greater following training in both groups.

Additionally, Seals and Chase (1989) reported a decreased forearm vascular resistance during lower body negative pressure following 30 weeks of vigorous aerobic training in middle aged men. They concluded that the training resulted in a higher maximal exercise capacity, but did not change baroreceptor reflex control of the heart. The mechanism of attenuated forearm vascular resistance following training is not known

2.3.3 Non-Neural Training Adaptations that Affect EPR

It is possible that the EPR itself does not change with training, but exercise training results in non-neural adaptations that reduce the stimulus that induces EPR activity. This may include skeletal muscle changes that reduce the amount of

metabolites produced during exercise, blood flow changes to the working muscle, or non-neural cardiovascular changes.

Skeletal muscle metabolic, morphologic, and histological changes may influence EPR stimulation during exercise. It is well known that exercise training results in an increase in mitichondrial volume, decreased blood lactate, and improved motor unit recruitment (Brooks et al., 1996). The magnitude of change from baseline in a given physiological variable is directly related to the intensity and amount of time the exercise is performed (Spina, 1999). The time course of these changes depends largely on the variable measured, but is generally accepted to take 7 to 14 days to see a significant change in muscle proteins and blood lactate (for a comprehensive review see Booth and Thomason, 1991).

Additionally, there is evidence that restricting blood flow to the muscle being trained can amplify the training effect. Using 4 weeks of leg exercise training with 50 mmHg of pressure on the leg, Sundberg, 1994 found a 20% higher in VO2peak, ~20% higher citrate synthetase concentration, 30% higher capillary to fiber ratio, and 10% higher glycogen concentration in the leg trained with low blood flow compared to no training. Previous work in afferent and efferent models have demonstrated that reducing blood flow to the working muscle is the most powerful stimulus for EPR activity and thus, should induce the largest magnitude change in EPR response to chronic activity.

Improvements in blood flow with exercise training may also influence the activity of the EPR. Vascular remodeling as a result of exercise training occurs at a very rapid rate. Several studies have shown that 4 weeks of training results in increased vessels

diameter in humans. Additionally, changes in NO production and vascular reactivity change in as little as 4 days (Laughlin, Oltman, Bowles, 1998).

Other non-neural cardiovascular adaptations to exercise may also influence the EPR response. Five months of long duration cycling training in young healthy men resulted in a decreased resting heart rate and increased VO_2max, left ventricle diameter, septal thickness, and left ventricular volume in diastole. These occurred without a significant change in heart rate variability (time and frequency domain) (Bonaduce, Petretta, Cvallaro, Apicella, Iannicieloo, Romano, et al., 1998). These findings indicate that a change in intrinsic rate occurred rather than an alteration in autonomic modulation.

There is limited evidence with forearm exercise to suggest that the EPR response to activity may change with exercise training. However, there is good evidence that the autonomic nervous system response to rest and activity alters following a period of exercise training. The contribution of the EPR to autonomic nervous system response both before and after training remains to be elucidated.

Additionally, the mechanism by which EPR alters with training is not well understood. Currently methodology has not been able to distinguish whether the change in EPR activity is due to alterations in the stimulus (metabolic or blood flow changes) or is an adaptation of the reflex itself (reduced receptor sensitivity). Better understanding of both the contribution of EPR to autonomic modulation during exercise and the mechanism by which the EPR response is modified will provide us with a better understanding of cardiovascular control.

2.4 Current Clinical Problems

Chronic changes in EPR sensitivity have also been documented to occur as a result of aging and chronic heart failure. These changes in EPR are associated with both decreased and increased EPR neural traffic and end organ response. Although little information exists concerning EPR response in older individuals, there is consistent evidence of decreased HRV and higher efferent sympathetic nerve activity at rest and during exercise (Schuit et al, 1999; Dinenno et al., 1999).

Additionally, there is evidence that blood flow is lower and heart rate response to the onset of exercise is slower, and oxygen consumption is reduced compared to younger individuals (Brooks et al., 1996; Dinenno et al., 1999). Another factor that may add to changes in EPR sensitivity is a loss of muscle mass and an increase in the percentage of slow twitch fibers in aging muscle (Brooks et al., 1996). Wilson, Dyke, Parsons, Wall, Pawelczyk, Williams, Mitchell. (1995) have shown that active fast twitch muscle has a higher EPR response than slow twitch muscle in rabbits. Therefore, it is possible that changes in EPR response may contribute to autonomic modulation changes seen in older individuals.

Considerably more literature exists linking EPR dysfunction with exercise intolerance in people with chronic heart failure. In addition to very high sympathetic nervous activity in people with heart failure both at rest and during exercise, peripheral adaptations to chronic heart failure may contribute to abnormal EPR activity (Zelis et al., 1991; Sverrisdottir et al., 2000).

One of the modifications of the skeletal muscle to heart failure is a reduction in the number of slow twitch and an increase in the number of fast twitch fibers.

Concomitantly, there is an overall reduction in the capacity for oxidative metabolism in skeletal muscles of the whole body. These changes result in greater lactate, potassium, and hydrogen ions all, which have a potent excitatory effect on GIII and GIV afferents in humans and animals (Zelis et al., 1991). Impaired blood flow to the working muscle as a result of impaired blood shunting, vascular dysfunction, and poor venous outflow may also contribute to an elevated EPR response to activity (Zelis et al., 1991).

Notarius, Atchison, Floras (2001) found a lower threshold and higher maximal activation for increase in MSNA with heart failure compared to controls during isometric and moderate intensity non-ischemic exercise. Piepoli et al. (1999) also found evidence of hyperactive EPR in patients with heart failure. In this study, all heart failure and control participants had an increase in systolic and diastolic blood pressure with post exercise occlusion, but magnitude of change was greater in people with more severe heart failure. Additionally, they found significant correlations among ventilatory response to post exercise occlusion, peak VO_2, and exercise intolerance. They concluded that EPR is hyperactive in patients with heart failure and may contribute to feeling of breathlessness and exercise intolerance.

Although the chronic adaptations to heart failure would suggest that EPR activity is elevated during exercise, there is some evidence that EPR is hypoactive in patients with heart failure. In agreement with studies named above, Negrao, Rondon, Tinucci, Alves, Roveda, Braga, et al. (2001) observed that MSNA at rest was related to disease severity. However, they also noted that MSNA and forearm vascular resistance change with mild and moderate isometric handgrip exercise was not different among groups (controls, mild heart failure, and severe heart failure). Sterns, Ettinger, Gray, Whisler,

Mosher, Smith, et al. (1991) found that MSNA remained high in controls during post exercise occlusion, but declined in patients with heart failure. Additionally, Negrao et al., (2001) observed that post exercise occlusion resulted in persistent elevation in MSNA and MAP in people with mild heart failure and controls, but in people with severe heart failure, MSNA and MAP returned to control values.

Therefore, considerable evidence exists to suggest that resting sympathetic activity to the periphery is high in people with heart failure and the magnitude of sympathetic activity is related to disease severity. However, some controversy exists concerning changes in sympathetic activity during exercise in people with heart failure. There is little disagreement that sympathetic activity is higher during exercise in heart failure compared to controls, but some studies have shown that the change from rest to exercise is not different from control participants. Information for post exercise forearm occlusion is also mixed and suggests that people with mild heart failure may have little to no hyperactivity in EPR response, but with more severe disease EPR may actually be hypoactive.

Investigations concerning the role of EPR in exercise intolerance and cardiovascular response in aging and heart failure needs further development. Establishment of exercise protocols as well as improved methods to study EPR contribution to autonomic modulation may aid in these studies. Additionally, the investigation of EPR as a contributor to exercise intolerance in other diseases such as peripheral vascular disease and diabetes should be advanced.

2.5 Conclusions and Future Directions

EPR appears to operate under a complex system of sensitivity to metabolites and muscular movement, which needs to be taken into account when studying cardiovascular control. Evidence suggests that multiple metabolites may be involved and related to the mode of exercise and the blood flow/metabolism mismatch involved. Further study concerning the conditions under which each metabolite(s) is most effective in stimulating EPR is vital to allow development of methodology to better isolate the EPR from other mechanisms of cardiovascular control. Additionally, better understanding of the receptor and neural pathways associated with EPR would allow development of pharmacological tools to intervene when EPR is hyper- or hypo-active.

EPR research is based on two basic sets of hypotheses. One of these sets of hypotheses is the central command versus the exercise pressor reflex hypotheses of cardiovascular control during exercise. The central command theory states that higher brain centers direct all cardiovascular control and motor unit recruitment during exercise, while the exercise pressor reflex theory states that peripheral mechanisms play an important role in cardiovascular control (Mitchell et al., 1983). Current knowledge about the EPR and other mechanisms of cardiovascular control cast significant doubt on the strict interpretation of the central command theory, however, the complex system of central command and peripheral cardiovascular control mechanisms is poorly understood.

Observations in afferent models suggest that parasympathetic withdrawal is mediated by EPR. However, evidence presented in efferent and end-organ models suggests that dynamic exercise has a higher degree of central command influence

compared to isometric exercise (Gonzales-Camerena et al., 2000; Illeamo et al., 1999; Seals and Victor, 1991). This conflict among models concerning the central command and exercise pressor reflex theories may be explained by the suggestion that EPR has a weak parasympathetic control aspect, which is usually overwhelmed by the more powerful central command. However, in afferent models (decerebrate cat/anesthetized animals) central command is eliminated or reduced which may allow this aspect of EPR to be expressed.

The other set of hypotheses influencing EPR literature is the traditional versus the emerging hypothesis of EPR function. The traditional hypothesis states that EPR only influences cardiovascular control under conditions where there is a mismatch between blood flow and skeletal muscle demand. The emerging hypothesis states that EPR is active and influencing cardiovascular control both during low intensity exercise and under conditions where blood flow and demand are not matched (Thomas et al., 1998). Data from isometric handgrip, dynamic handgrip, arm cycling and isometric leg extension suggest that restricting blood flow to the working muscle lowers the threshold for increasing sympathetic activity and increases the magnitude of the cardiovascular response for a given intensity of activity and supports the traditional hypothesis (Saito et al., 1986; Victor et al., 1987; Seals, 1989; Victor and Seals, 1989).

Afferent models (decerebrate cat) support the emerging hypothesis that low intensity dynamic exercise also induces EPR activity. However, in humans the threshold for inducing EPR activity appears to be higher. Efferent and end-organ models suggest that EPR is most active under conditions where blood flow is restricted for a period of time (occlusion or isometric exercise), but is active under conditions of

high intensity (60%MVC or greater) resisted exercise in the forearm and low intensity resisted exercise in the leg and upper body (30%MVC or higher). Therefore, in an intact subject, the traditional hypothesis of EPR activity is partially supported in that the relative contribution of EPR is related to the degree of blood flow restriction in the working muscle. However, EPR activity is also related to the size of the muscle mass used, and the amount of resistance to movement supplied by the activity, which somewhat supports the emerging hypothesis.

Therefore, different models for evaluating EPR support the basic theoretical framework of the current hypotheses. Future research should focus on developing models of EPR function that are non-invasive and involve minimal interference with other cardiovascular control mechanisms. Additionally there is a need to move EPR research to more functionally significant models such as, lower limb exercise.

There is very little known concerning the chronic adaptations of the EPR. However, there is significant evidence that a change in autonomic modulation does occur with exercise training. Before we can understand how to intervene in clinically significant changes in EPR, we need to know if EPR is modifiable through training and/or pharmacology. Improved understanding of the mechanism of change in autonomic modulation with training and the adaptability of EPR will allow development of treatment strategies for clinical populations effected by hyperactive EPR.

CHAPTER 3 MATERIALS AND METHODS

3.1 Participants

Volunteers (age 18 to 30 years of age) were asked to participate in this study. The sample size estimate for this study was based on a desired type I error rate of 5% and type II error rate of 20% and for a longitudinal study with a control group. The calculation for the estimate made use of the effect size (ES), which was calculated as the smallest difference worth detecting divided by the expected standard deviation of the parameter of interest. The calculation also made use of the test-retest reliability of the parameter (r).

The primary variable of interest was the natural logarithm of normalized units for low frequency power (LFnu). Therefore, we estimated the sample size based on the expected behavior of this parameter. While the effect of exercise training on a reactivity measure, in general, has been documented in our laboratory, the magnitude of change in this variable following dynamic handgrip exercise was not known. Therefore, we also estimated the sample size based on the expected values for reactive hyperemic blood flow (RHBF). Our laboratory has previously documented training adaptations in RHBF following handgrip exercise training. We used the most conservative of the two estimates for the determination of the sample size for the study.

Estimating sample size using LFnu (Lee, 2001; Kluess et al., 2001):

ES = smallest difference to detect / standard deviation = 0.3units / 0.5 units = 0.6

$N = 32 / ES^2 = 32 / 0.6^2 = 89$

n(total) = 2N(1-r)

r for LFnu = 0.87

n = 178 (0.13) = 23.1 = 12 per group

Estimating sample size using reactive hyperemic blood flow (RHBF)(Alomari et al, 2001):

Effect size = smallest difference to detect / standard deviation = 5units / 8 units = 0.63

$N = 32 / ES^2 = 32 / .63^2 = 80$

n(total) = 2N(1-r)

r for RHBF = 0.93

n = 160 (0.07) = 11.2 = 6 per group

All volunteers were healthy adults without any overt signs such as diabetes, heart disease, hypertension, emphysema, gastrointestinal disturbances or bleeding or gynecological problems. Individuals with any current medical problems (including those listed above), current infection, or on medication known to affect cardiovascular function were excluded from the study (see appendix B for the medical history questionnaire). Additionally, volunteers that had taken aspirin within two weeks prior to testing were excluded.

3.2 Design

This study involved an experimental design and made use of a mixed-model ANOVA to test for main effects of treatment and test condition as well as treatment by condition interaction. There were two levels of treatment (trained arm vs. control) and two levels of conditions (pre versus post). Additionally, the effect of the treatment was tested against two controls (internal control, i.e. dominant arm, and an external control, i.e., untrained non-dominant arms of age and gender matched participants). Thus the study made use of two mixed-model ANOVAS, one including an internal control, and

one including an external control. A more complete description of the statistical comparisons is given in tables 3.1 to 3.6.

3.3 Procedures

Participants reported to the laboratory on two occasions for testing. All visits were held prior to 11AM and participants were asked to refrain from eating, drinking, and exercising at least 12 hours prior to the test.

3.3.1 Session 1

Upon arriving at the laboratory, participants were asked some general questions about their health history in order to assess for the presence of any disease, condition, or medical therapy that might affect cardiovascular function.

The circumference of both forearms was measured using a measuring tape at quarter distances from the midolecranon process to the ulnar styloid (sites 1-4, respectively). While lying in a supine position, the participant was asked to squeeze the handgrip dynamometer attachment (Biodex Medical Systems, Shirley, New York) as hard as they could for 5-seconds. They were allowed three trials with 30 seconds in between each trial. The three MVC's were averaged and multiplied by 0.60 to derive the torque used during the exercise condition (60% MVC). The participant was then instrumented with equipment to record blood flow, respiratory rate, ECG, and blood pressure (see diagram C.1 in appendix C for a diagram of instrumentation for testing the right arm).

An EC-5R plethysmography system (Hokanson, 1995) with a mercury-in-silastic strain gauge and blood pressure cuffs on the arm and wrist were used to obtain forearm blood flow measurements. Blood pressure cuffs were positioned around the participant's

arm and wrist on the side to be exercised, and a mercury-in-silastic strain gauge placed around the forearm approximately 10 cm distal to the olecranon process for measurement of resting and post-occlusion blood flow. The forearm was extended, slightly supinated, and supported by a Styrofoam block. Baseline blood flows were recorded at a paper speed of 5 centimeters/second and reactive hyperemia blood flow (RHBF) was recorded at a paper speed of 25 centimeters/second. Additionally, a pneumotachometer was secured around the participant's torso and interfaced with the Biopac MP100 to measure respiratory frequency during the protocol.

Heart rhythm was continuously collected using a 3-lead ECG interfaced with a Biopac MP100 and its companion software Acqknowledge (model MP100A, Biopac Inc., Santa Barbara, CA). All data was collected at 200hz. Lastly, the non-working arm was fitted with a blood pressure cuff on the arm and a Colin 7000 tonometric sensor (Colin medical instruments, San Antonio, TX) on the wrist to measure blood pressure continuously throughout the protocol. This was also interfaced with the Biopac MP100 and the data was collected at 200 hz. For a visual representation of the set-up for testing the right arm see appendix C diagram C.1.

Baseline forearm blood flow measurements were obtained from the arm to be exercised. Immediately before the blood flow measurements, hand circulation was occluded for 1 minute by inflating the cuff at the wrist to 240 mmHg. The arm cuff was then inflated to 50 mmHg and the baseline blood flow measurement was recorded. Following the resting blood flow measure, the arm cuff was inflated to 200 mmHg for 3 minutes. One minute prior to release of the arm cuff, the wrist cuff was inflated to 240 mmHg. RHBF was recorded at an arm pressure of 50 mmHg.

The participant was then asked to perform a handgrip exercise task preceded by 5 minutes of quiet rest (spontaneous breathing, SB1). The handgrip exercise task consisted of squeezing a handgrip-measuring device once every two seconds at 60% MVC while the cuff on the arm was inflated to 50 mmHg for 5 minutes (H60+50mmHg). The participant had visual feedback regarding the amount of force they were exerting and an auditory cue for the cadence. Additionally, they received verbal encouragement to maintain the force and cadence.

Within the last 4 seconds of the exercising condition, the arm cuff was inflated to 200 mmHg for 3 minutes (FAO). Lastly, a blood flow measurement was obtained by inflating the wrist cuff one-minute prior to the release of the arm cuff. The arm cuff pressure was reduced to 50 mmHg and a blood flow measurement recorded. Throughout the testing procedures, heart rate and blood pressure were recorded. The testing procedures described above were repeated using the opposite arm as the working arm. The testing procedures for both arms lasted approximately 150 minutes. For a visual representation of the protocol time line see appendix C diagram C.2.

Following testing, each participant was randomized to either non-dominant arm exercise training (training group) or the no training group. The no training group (external control group) refrained from activities that specifically exercised the forearm muscles for 4 weeks.

3.3.2 Exercise Training

Participants reported to room 20 in the Gym Armory 4 days per week for 4 weeks. Each day, the participant's resting maximal voluntary contraction in the non-dominant arm was measured using a Lafayette instrument handgrip dynamometer. The

participant was asked to perform a handgrip exercise for 20 minutes with their non-dominant arm. The handgrip exercise task consisted of squeezing a handgrip-measuring device once every two seconds at 60% of their resting MVC. Participants were required to have an attrition rate of no less than 85%.

3.3.3 Session 2

Following 4 weeks of exercise training or the control period the participants repeated the protocol outlined in session 1.

3.4 Data Treatment

3.4.1 Blood Flow Analysis

Blood flow was calculated as the rate of arterial inflow and reported in milliliters per minute per 100 milliliters of tissue (ml/100 ml/min). Additionally, the change in blood flow from baseline to RHBF was calculated (delta BF=RHBF-baseline). Vascular resistance was also calculated for the baseline and RHBF conditions as MAP/ blood flow. The MAP collected during the SB1 condition was used for this calculation (taken approximately 90 seconds after the RHBF blood flow measurement).

3.4.2 Pneumotachometer Data Analysis

Pneumotachometer data was analyzed using Acqknowledge 3.0 software. Respiratory rate was obtained by counting the number of breathing cycles in a 15 second period and multiplying this number by 4 to report the data in breaths per minute. The 15-second period was within the data segment described below for ECG and blood pressure wave analysis.

For pre and post comparisons the data was expressed as an absolute change in respiratory rate from spontaneous breathing values. Absolute change was calculated

by subtracting the value obtained during exercise or forearm occlusion from values obtained during spontaneous breathing (absolute change with exercise = value during exercise - value during spontaneous breathing; absolute change with forearm occlusion = value during forearm occlusion - value during spontaneous breathing).

3.4.3 ECG Analysis

The ECG data was analyzed using Acqknowledge 3.0 software. The ECG data was visually inspected for non-sinus beats and converted to a tachogram of R-R period. A custom made program in Matlab was used to analyze 2.2-minute segments of tachogram data for frequency and time domain parameters. Tachogram segments were taken from a representative section of data at least 90 seconds into the data collection of spontaneous breathing and H60+50mmHg; and sampled from the first 2.5 minutes of FAO.

Raw data was reported as mean heart period (mean R-R), standard deviation of the normal sinus R-R intervals (SDNN), low frequency power (LF: 0.04 hz to 0.15 hz) and high frequency power (HF: 0.15 hz to 0.40 hz). The frequency domain was additionally reported in normalized frequency units (LFnu: power / sum of LF + HF). For pre and post comparisons the data was expressed as an absolute change in Mean R-R, SDNN, LFnu from spontaneous breathing values. The formula was absolute change with exercise (ΔH60+50mmHg) = value during exercise - value during spontaneous breathing and absolute change with forearm occlusion (ΔFAO) = value during forearm occlusion - value during spontaneous breathing).

3.4.4 Blood Pressure Analysis

The blood pressure wave data was be analyzed using Acqknowledge 3.0 software. Mean arterial pressure (MAP) was obtained by taking the mean of a 2.2-minute blood pressure wave segment taken after the first 90 seconds of recording of spontaneous breathing and H60+50mmHg. The 2.2-minute segment for FAO was obtained during the first 2.5-minutes of the recording.

Using the segments described above, the data was also analyzed for the mean of the maximum blood pressure wave peak (systolic blood pressure, SBP) and the mean of the minimum blood pressure wave peak (diastolic blood pressure, DBP). For a diagram of the parts of a blood pressure wave see appendix C diagram C.3.

For pre and post comparisons the data was expressed as an absolute change in the condition (H60+50mmHg or FAO) from spontaneous breathing values. Absolute change was calculated by subtracting the value obtained during H60+50mmHg (ΔH60+50mmHG) or forearm occlusion (ΔFAO) from values obtained during spontaneous breathing (ΔH60+50mmHG = value during H60+50mmHg - value during spontaneous breathing; (ΔFAO = value during FAO - value during spontaneous breathing).

3.5 Statistical Analysis

3.5.1 Participant Characteristics and Blood Flow

A one-way ANOVA was used to evaluate pre-test between group differences in age, height, weight. Additionally a one-way ANOVA was used to evaluate pre-test differences in the non-dominant arm between groups in arm circumference (sites 1-4), MVC, baseline blood flow, RHBF, delta blood flow, and vascular resistance. A one-way

repeated measures ANOVA was used to evaluate pre-test differences between arms (dominant versus non-dominant) in the Training group for arm circumference (sites 1-4), MVC, baseline blood flow, RHBF, and delta blood flow. The effect of the treatment between groups were evaluated in arm circumference and MVC using a 2 x2 mixed model ANOVA for arm circumference (sites 1-4), MVC, baseline blood flow, RHBF, and delta BF (See table 3.1).

Table 3.1: Statistical comparison for treatment effect between groups using raw data

2 levels of factor	Pre	Post
Non-dominant external control group	Raw data	Raw data
Non-dominant Training group	Raw data	Raw data

The effect of the treatment within the training group was evaluated in arm circumference, MVC, and blood flow using a 2 x2 mixed model ANOVA for arm circumference (sites 1-4), MVC, baseline blood flow, RHBF, and delta blood flow. See table 3.2.

Table 3.2: Statistical comparison for treatment effect within groups using raw data

2 levels of factor	pre	post
Non-dominant arm	Raw data	Raw data
Dominant arm	Raw data	Raw data

3.5.2 Respiratory Rate, HRV, and Blood Pressure

A 3x2 mixed-model ANOVA was used to evaluate pre-test differences among the conditions (SB1, H60+50mmHg, FAO) within groups in respiratory rate, mean R-R,

SDNN, LFnu (statistics were run using the natural log of LFnu due to the non-normal characteristics of the data), MAP, SBP, and DBP (table 3.3 and 3.4).

Table 3.3: Statistical comparison for the effect of condition in the Training group on pre data only.

3 condition (repeated measures)			
2 levels of factor	SB1	H60+50mmHg	FAO
Non-dominant	Raw data	Raw data	Raw data
Dominant	Raw data	Raw data	Raw data

Table 3.4: Statistical comparison for the effect of condition in the external control group on pre-test data only.

3 condition (repeated measures)			
2 levels of factor	SB	H60+50mmHg	FAO
Non-dominant	Raw data	Raw data	Raw data
Dominant	Raw data	Raw data	Raw data

A 2x2 mixed model ANOVA was used to evaluate the within group treatment effect using ΔH60 and ΔFAO for respiratory rate, mean R-R, SDNN, LFnu, MAP, SBP, and DBP (table 3.5).

Table 3.5: Statistical comparison for within group treatment effect using change in absolute values from SB1 to H60+50mmHg or FAO

2 conditions (repeated measures)		
2 levels of factor	Pre	Post
Non-dominant arm	ΔH60 or ΔFAO	ΔH60 or ΔFAO
Dominant arm	ΔH60 or ΔFAO	ΔH60 or ΔFAO

Additionally, the treatment effect between groups was evaluated using a 2x2 (pre/post, non-dominant training group arm /non-dominant external control arm) mixed ANOVA using ΔH60 and ΔFAO for respiratory rate, mean R-R, SDNN, LFnu, MAP, SBP, and DBP (table 3.6).

Table 3.6: Statistical comparison for treatment effect between groups using change in absolute values from SB1 to H60+50mmHg or FAO

2 levels of factor	Pre	Post
Non-dominant training group	ΔH60 or ΔFAO	ΔH60 or ΔFAO
Non-dominant external control	ΔH60 or ΔFAO	ΔH60 or ΔFAO

Alpha was set a priori at 0.05. A Tukey's protected T-test was used for post hoc analysis. For the variables LFnu and MAP during ΔH60 and ΔFAO, post hoc analyses were performed to determine the observed type II error rate for the main effect of group, treatment, and the interaction. These are found in Appendix F.

3.5.3 Associations Among Variables

We evaluated the pre-test data for relationships among forearm circumference (site 1) and the absolute magnitude of change in the cardiovascular parameters (as compared to the SB1 condition, i.e., ΔH60+50mmHg and ΔFAO) for the variables Mean R-R interval, SDNN, LFnu, and MAP. We used linear regression modeling employing the least squares technique. Alpha was set a priori at 0.05.

CHAPTER 4 RESULTS

4.1 Participant Characteristics

21 men and 19 women were recruited for participation in this study. Nineteen participants were assigned to the external control group and 21 were assigned to the training group. Over the course of the study, 3 participants were removed from the control group (1 woman and 2 men) and 2 participants were removed from the training group (2 men) due to non-compliance with the pre-test instructions or non-compliance with the training protocol. There were no significant differences in participant characteristics between non-compliers and those who completed the study. Table 4.1 summarizes the final participant characteristics (taken at pre-test) for the groups.

Table 4.1: Participant characteristics

	External control (n=16) Non-dominant	Training (n=19) Non-dominant	Training (n=19) Dominant
Men/Women	8/8	9/10	
Age	20.07±1.75	20.63±0.83	
Height (inches)	67.80±2.91	67.47±4.76	
Weight	161.53±28.57	168.89±34.07	
Arm circ site 1 pre(cm)	26.10±2.64	26.58±3.31	27.21±3.30
Arm circ site 2 pre	23.30±2.37	23.13±3.32	24.26±3.35
Arm circ site 3 pre	18.87±2.08	18.86±2.17	18.91±2.21
Arm circ site 4 pre	16.28±1.24	16.68±1.56	16.45±1.37
MVC pre (peak torque)	69.10±24.15	71.77±35.72	78.90±33.90

The external control and training group were well matched, inasmuch as there were no group differences in age, height, weight, non-dominant arm circumferences, and MVC. In addition there were no differences between dominant and non-dominant arm circumferences, and MVC within the training group.

4.2 EPR effect (pre-data only)

4.2.1 Respiratory Rate, HRV, and Blood Pressure

There were no main effects of group or test condition (SB1, H60+50mmHg, and FAO) on respiratory rate (see figure 4.1). However, there was a group by condition interaction such that respiratory rate was elevated above SB1 and FAO during H60+50mmHg, but only in the Training group during non-dominant arm exercise (H60+50mmHg = 19.58±5.56 vs. SB1 = 16.76±3.15 and FAO = 16.56±5.85

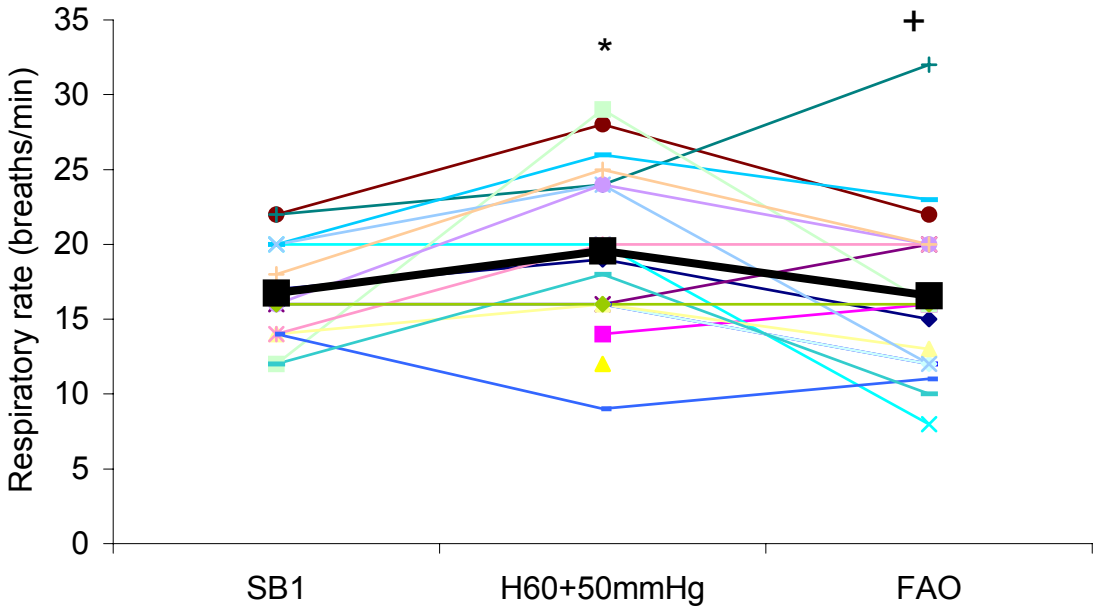

Figure 4.1: Individual responses for respiratory rate to the conditions in the non-dominant arm of the training group (pre-test). The bold line indicates the mean of the group. * p<0.05 from SB1; +p<0.05 from H60+50mmHg

breaths/min; $p<0.05$). Consequently, respiratory rate was used as a covariate in subsequent analyses of pretest data (Mean RR, SDNN, LFnu). There was no main effect of group on Mean R-R, SDNN, LFnu, MAP, SBP, or DBP. However, there was a main effect of test condition on Mean R-R, SDNN, LFnu, MAP, SBP, and DBP. Figure 4.2 to 4.4 represents the individual responses in the training group in the non-dominant arm pre-training to SB1, H60+50mmHg, and FAO for the variables mean R-R, LFnu, and MAP.

Post-hoc tests revealed that SDNN decreased during H60+50mmHg and returned to baseline with FAO for all groups. See table 4.2 (H different from H60+50mmHg and * different from SB1). Likewise, LFnu and SBP increased during H60+50mmHg and returned to baseline during FAO for all groups (see table 4.3 for SBP results; H different from H60+50mmHg and * different from SB1).

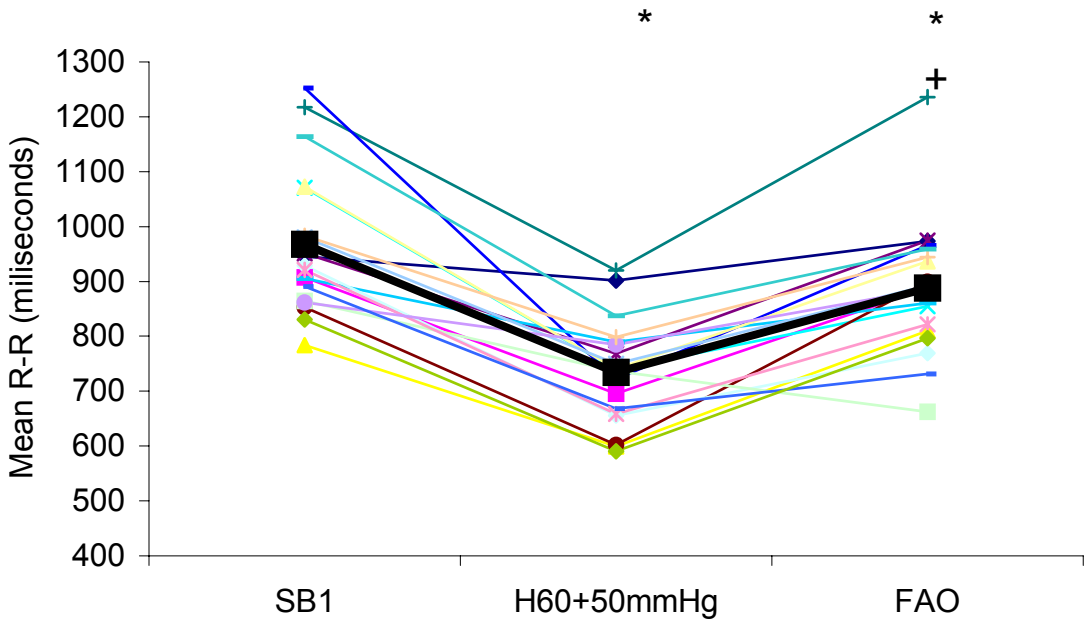

Figure 4.2: Individual responses for mean R-R to the conditions in the non-dominant arm of the training group (pre-test). The bold line indicates the mean of the group. * $p<0.05$ from SB1; +$p<0.05$ from H60+50mmHg

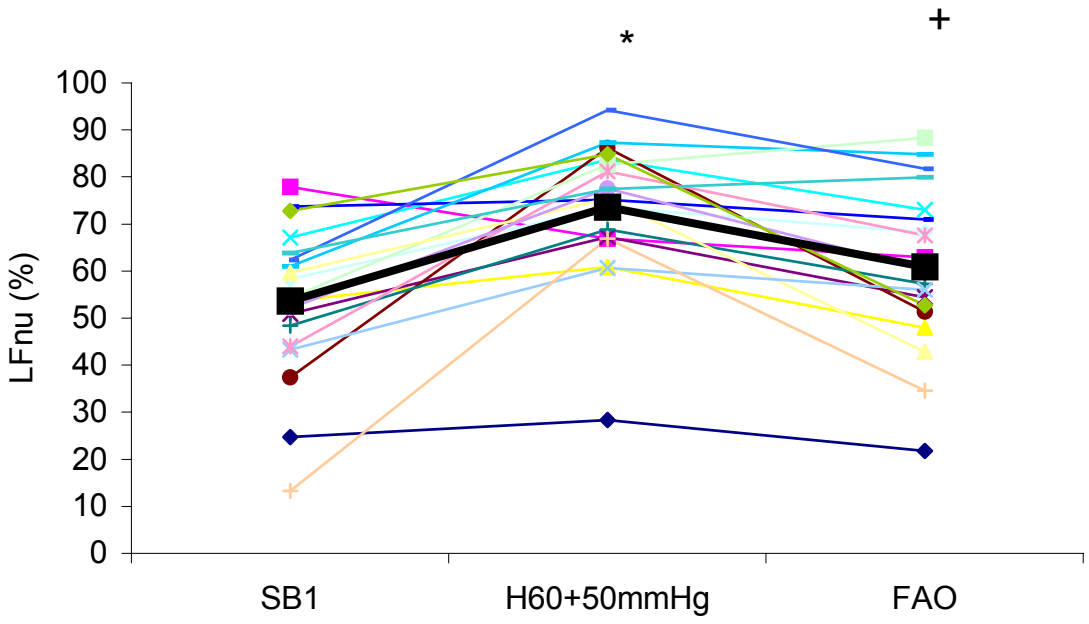

Figure 4.3: Individual responses for LFnu to the conditions in the non-dominant arm of the training group (pre-test). The bold line indicates the mean of the group. * p<0.05 from SB1; +p<0.05 from H60+50mmHg

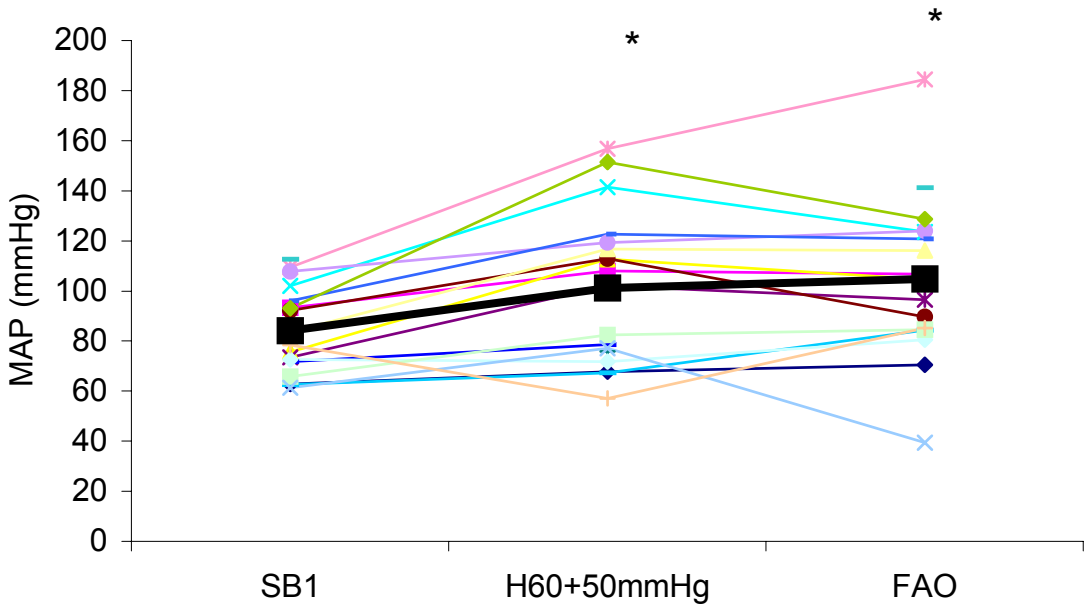

Figure 4.4: Individual responses for MAP to the conditions in the non-dominant arm of the training group (pre-test). The bold line indicates the mean of the group. * p<0.05 from SB1; +p<0.05 from H60+50mmHg

Table 4.2: The effect of condition on SDNN by group and arm (pre-test only).

SDNN (miliseconds)	External control (n=16) Non-dominant	Training (n=19) Non-dominant	Training (n=18) Dominant
SB1	88.62±41.94	90.85±40.46	85.76±25.70
H60+50mmHg	45.69±20.41*	46.96±15.07*	49.84±30.87*
FAO	117.51±80.84H	87.14±42.02H	98.15±47.30H

Mean±standard deviation

Table 4.3: The effect of condition on SBP by group and arm (pre-test only).

SBP (mmHg)	External control (n=16) Non-dominant	Training (n=19) Non-dominant	Training (n=20) Dominant
SB1	122.80±22.88	125.29±28.27	117.55±24.41
H60+50mmHg	150.39±29.34*	142.77±43.56*	142.80±30.85*
FAO	137.61±32.87	141.22±43.65	137.61±24.28

Mean±standard deviation

In the case of MAP, however, the post-hoc tests indicated that the values increased during H60+50mmHg and remained elevated during FAO (see figure 4.4). The main effects on mean R-R and DBP are modified by the appearance of group by test condition interactions (see table 4.4 for DBP results; H different from H60+50mmHg and * different from SB1).

Similar to SDNN, the values for Mean R-R were lower during H60+50mmHg as compared to SB1 for all groups (see figure 4.2). However, while the values tended to

Table 4.4: The effect of condition on DBP by group and arm (pre-test only).

DBP (mmHg)	External control (n=16) Non-dominant	Training (n=19) Non-dominant	Training (n=20) Dominant
SB1	69.69±25.10	68.54±18.52	56.82±19.37
H60+50mmHg	86.96±22.23*	76.39±27.07*	76.86±21.18*
FAO	77.50±27.70	79.52±30.24*	71.25±22.92*

Mean±standard deviation

return to baseline (SB1) during FAO, only the external controls returned completely to baseline. With respect to DBP, the values increased during H60+50mmHg, and tended to return to baseline during FAO, but only completely recovered in the external control group.

4.2.2 Associations Among Variables

Additionally, we chose to evaluate the pre-test data for relationships among forearm circumference (site 1) and the absolute magnitude of change in the cardiovascular parameters (as compared to the SB1 condition), i.e., ΔH60+50mmHg and ΔFAO for the variables Mean R-R interval, SDNN, LFnu, and MAP. We found no associations between circumference site and the Δconditions for the variables SDNN or LFnu. However, ΔH60+50mmHg for the variable mean R-R was associated (Dominant arm (D): $p=0.03$, $R^2=0.14$; non-dominant arm (ND): $p=0.001$, $R^2=0.26$) with forearm circumference (slope: D=-14.79 msec*cm, ND=-22.11 msec*cm), where a larger decrease in mean R-R from SB1 to H60+50mmHg was associated with a larger arm circumference. See figure 4.5 for a scatter plot of the data with the regression line.

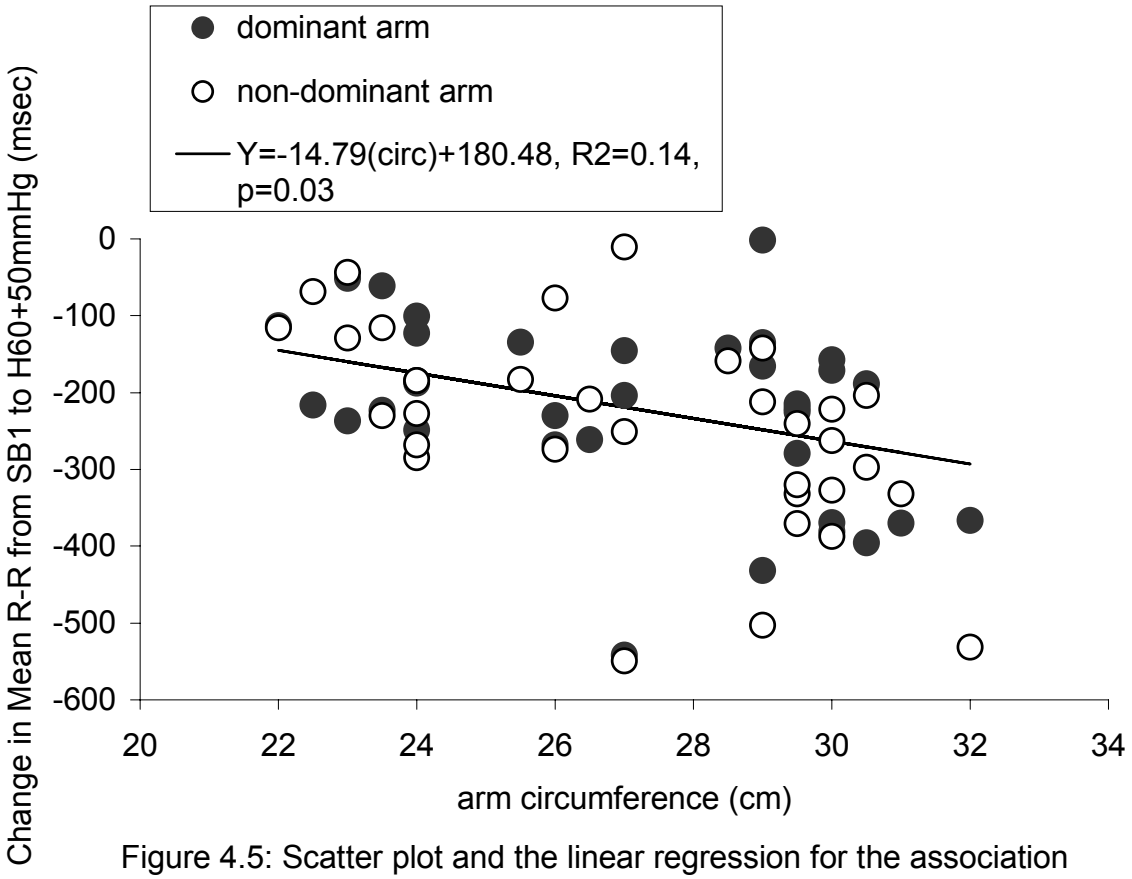

Figure 4.5: Scatter plot and the linear regression for the association between forearm circumference and the change in Mean R-R from SB1 to H60+50mmHg.

There was also a significant association between ΔFAO for mean R-R to forearm circumference with (ND: p=0.03, R^2=0.13; slope=14.11msec*cm), where mean R-R recovered to baseline levels more quickly with a larger arm circumference. See figure 4.6 for a scatter plot of the data with the regression line.

Additionally, there was a significant positive correlation (slope=2.17 mmHg*cm) between the ΔH60+50mmHg for MAP (dominant arm) and forearm circumference (p=0.04, R^2=0.13). See figure 4.7. ΔFAO for MAP was also associated with (slope: D=-2.11 mmHg*cm) forearm circumference (D: p=0.03, R^2=0.15). See figure 4.8 for a scatter plot of the data with the regression line included.

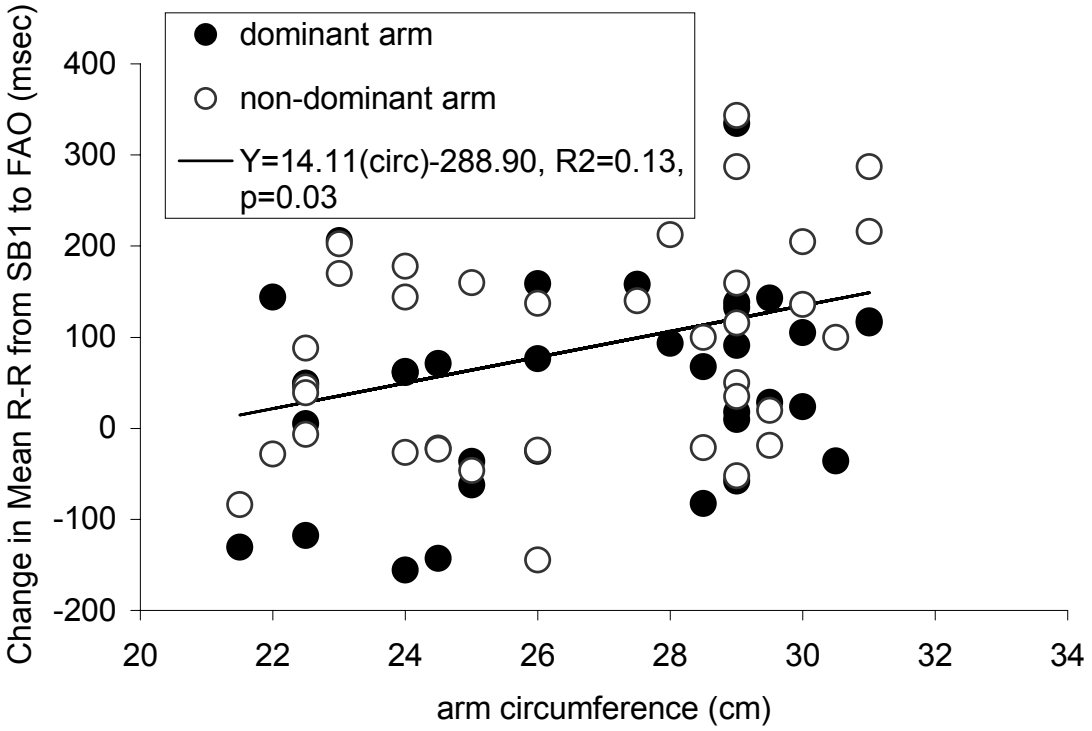

Figure 4.6: Scatter plot and the linear regression for the association between forearm circumference and the change in Mean R-R from SB1 to FAO.

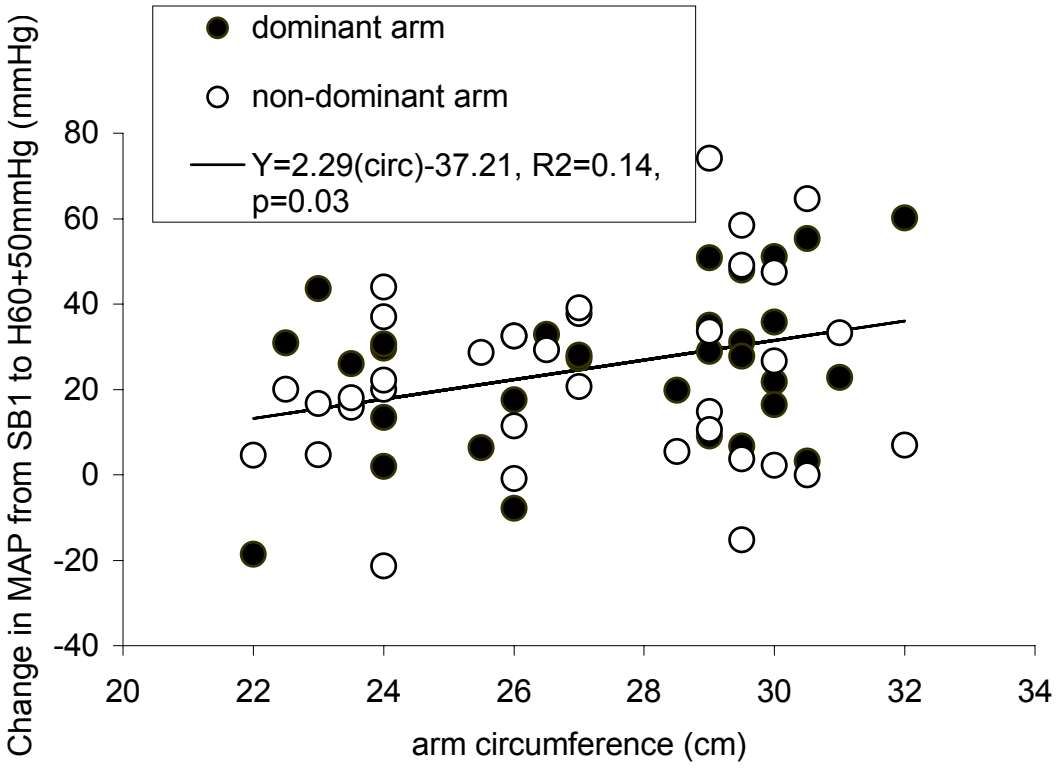

Figure 4.7: Scatter plot and linear regression for the association between forearm circumference and the change in MAP from SB1 to H60+50mmHg.

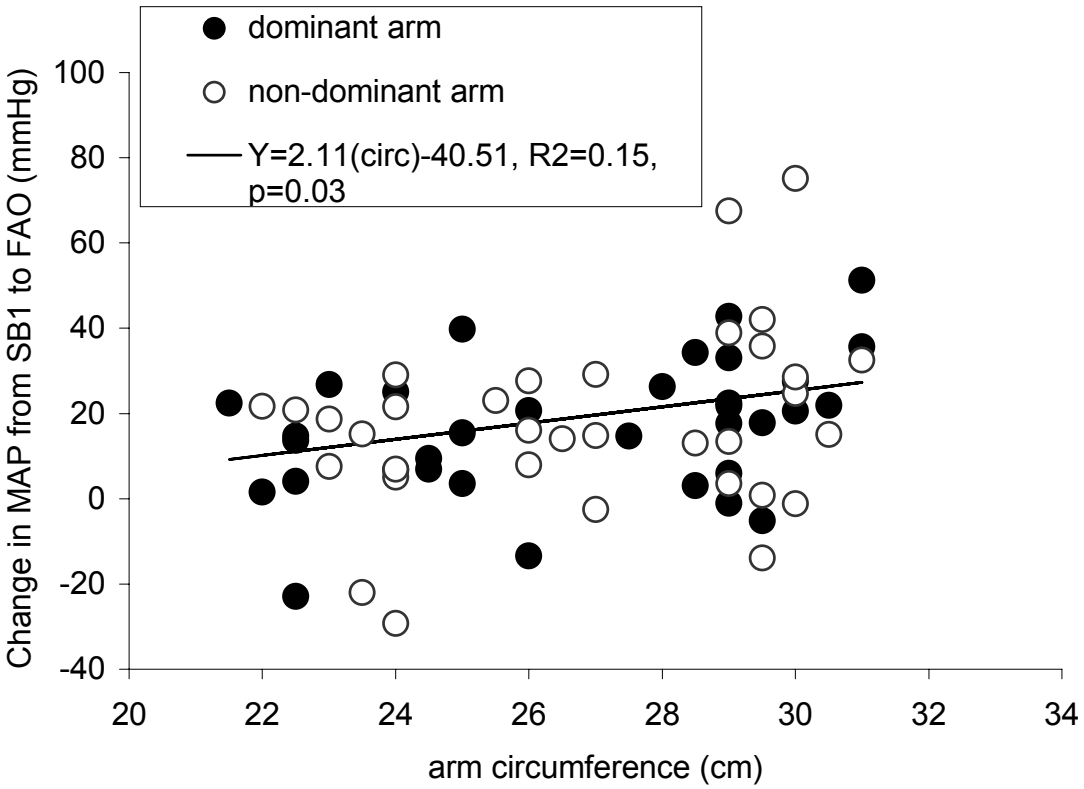

Figure 4.8: Scatter plot and the linear regression for the association between forearm circumference and the change in MAP from SB1 to FAO.

4.3 Effect of Training

4.3.1 Participant Characteristics

There were no main effects of treatment period or group by treatment period interactions on arm circumference at any site or handgrip MVC. Data is summarized in table 4.5.

4.3.2 Blood Flow and Vascular Resistance

The results of the mixed-model ANOVA revealed no main effect of treatment or group by treatment interactions for any of the blood flow or vascular resistance parameters (baseline, or during reactive hyperemia). However, when a paired T-test was run on the non-dominant arm of the training group (i.e., the trained arm) there was a significant decrease in vascular resistance during RHBF with training (p=0.03). A

summary of individual response to the control condition in the dominant arm in figure 4.9, and training in the non-dominant arm is in figure 4.10.

Table 4.5: The effect of treatment for arm circumference and MVC

		External control (n=16) Non-dominant	Training (n=19) Non-dominant	Training (n=19) Dominant
Arm circ site 1 (cm)	pre	26.10±2.64	26.58±3.31	27.21±3.30
	post	26.10±2.74	26.63±3.32	27.21±3.30
Arm circ site 2 (cm)	pre	23.30±2.37	23.13±3.32	24.26±3.35
	post	23.50±2.98	23.42±3.22	24.26±3.35
Arm circ site 3 (cm)	pre	18.87±2.08	18.86±2.17	18.91±2.21
	post	18.83±2.13	18.58±2.13	18.91±2.21
Arm circ site 4 (cm)	pre	16.28±1.24	16.68±1.56	16.45±1.37
	post	16.20±1.08	16.53±1.56	16.45±1.37
MVC post (peak torque)				
	pre	69.10±24.15	71.77±35.72	78.90±33.90
	post	70.99±23.50	75.80±31.10	77.47±29.61

Mean±standard deviation

4.3.3 Respiratory Rate, HRV, and Blood Pressure

Two separate 2 x 3 mixed model ANOVAs were conducted to examine main effects of group and treatment period and group by treatment period interactions on the ΔH60+50mmHg and the ΔFAO for the variables respiratory rate, Mean R-R interval, SDNN, LFnu, MAP, SBP, and DBP. One ANOVA included the internal control

(dominant arm of the training group), while the other employed the external control (Non-dominant arm of external control group).

The results of the ANOVAs, in either case, revealed no main effects of group nor treatment period, nor group by treatment period interactions on any of the Δconditions for the parameters indicated. The means and standard deviations for the groups are summarized in tables 4.6 to 4.12.

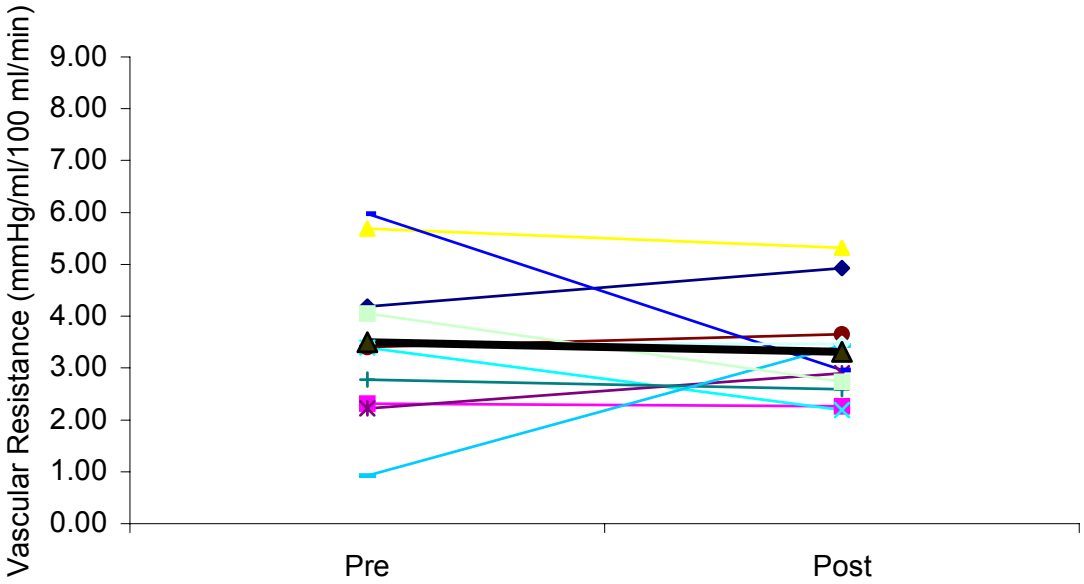

Figure 4.9: Individual responses for the change in vascular resistance during RHBF before (pre) and after (post) the control period in the dominant arm of the training group.

Figure D.1 to D.3 in appendix D represents the individual responses in the Training group in the non-dominant arm during FAO pre-training and post training for Mean R-R, LFnu, and MAP. Additionally, post-test absolute values for SB1, H60+50mmHg, and FAO are summarized in tables E.1 through E.7 in appendix E.

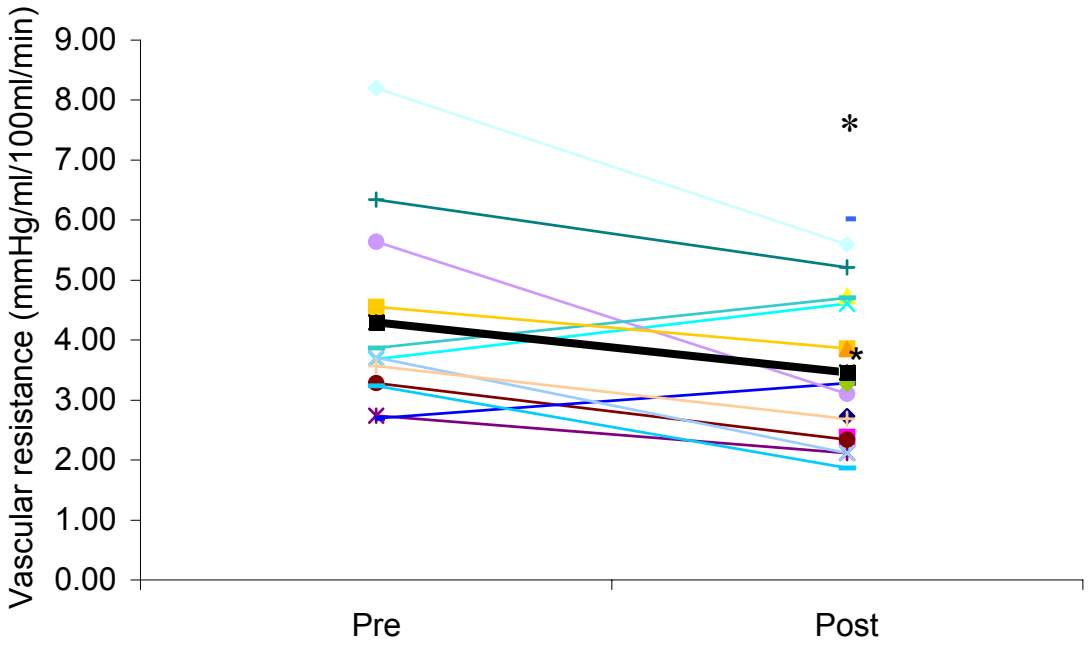

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Figure 4.10: Individual responses for the change in vascular resistance during RHBF before (pre) and after (post) training in the non-dominant arm of the training group. * p=0.03 using a paired T-test different from pre-test.

Table 4.6: The effect of treatment on respiratory rate by group and arm.

respiratory rate (breaths/min)		External control (n=14) Non-dominant	Training (n=17) Non-dominant	Training (n=17) Dominant
ΔH60+50mmHg	pre	2.42±6.23	3.59±4.87	4.26±6.51
	post	3.50±6.36	4.00±5.34	3.53±5.17
ΔFAO	pre	-0.50±4.25	0.18±5.33	1.00±4.49
	post	2.07±4.01	1.41±4.73	0.78±4.86

Mean±standard deviation

Table 4.7: The effect of treatment on mean R-R by group and arm

mean R-R (msec)		External control (n=15) Non-dominant	Training (n=19) Non-dominant	Training (n=19) Dominant
ΔH60+50mmHg	pre	-255.48±153.64	-240.83±112.31	-229.63±102.62
	post	-221.33±97.34	-236.18±146.03	-223.52±131.00
ΔFAO	pre	75.21±119.43	93.38±115.35	52.83±135.83
	post	59.74±96.28	83.44±109.70	35.95±136.90

Mean±standard deviation

Table 4.8: The effect of treatment on SDNN by group and arm

SDNN (msec)		External control (n=15) Non-dominant	Training (n=19) Non-dominant	Training (n=19) Dominant
ΔH60+50mmHg	pre	-41.63±39.51	-47.80±42.30	-34.95±40.36
	post	-52.43±42.59	-41.00±34.19	-49.60±33.61
ΔFAO	pre	-29.23±76.91	6.29±27.45	12.25±103.17
	post	-2.39±53.17	-9.23±30.27	-9.87±39.80

Mean±standard deviation

While there were no statistically significant main or interaction effects noted, there appeared to be a trend toward a treatment by group interaction for SDNN (p=0.08). The mean decrease in SDNN observed in the trained arm was of a smaller magnitude after the training period, whereas in the internal and external control arms, the mean decrease in SDNN was of a greater magnitude following training (see table 4.8). We also found a trend toward a main effect of treatment in SBP (p=0.08), where

both groups (non-dominant arm) decreased ΔH60+50mmHg from pre- to post-test (see table 4.11). Lastly, we found a trend towards a treatment by arm interaction with ΔH60+50mmHg in DBP (p=0.08). In the training group, DBP increased from pre- to post-test in the non-dominant arm, but decreased in the dominant arm with ΔH60+50mmHg (see table 4.12).

Table 4.9: The effect of treatment on LFnu by group and arm.

LFnu (%)		External control (n=15) Non-dominant	Training (n=19) Non-dominant	Training (n=19) Dominant
ΔH60+50mmHg	pre	18.71±14.04	19.33±15.59	11.74±16.24
	post	13.57±17.66	20.74±19.97	8.66±23.39
ΔFAO	pre	4.63±20.85	-6.66±14.72	2.03±18.70
	post	-1.61±18.27	-6.16±17.40	2.83±20.78

Mean±standard deviation

Table 4.10: The effect of treatment on MAP by group and arm

MAP (mmHg)		External control (n=14) Non-dominant	Training (n=19) Non-dominant	Training (n=16) Dominant
ΔH60+50mmHg	pre	30.42±22.06	19.26±19.42	23.20±20.09
	post	17.47±12.17	19.42±13.93	16.99±13.22
ΔFAO	pre	19.44±21.53	18.74±20.09	12.89±21.30
	post	15.09±13.16	14.06±19.52	10.26±20.73

Mean±standard deviation

Table 4.11: The effect of treatment on SBP by group and arm

SBP (mmHg)		External control (n=14 Non-dominant	Training (n=18) Non-dominant	Training (n=16) Dominant
ΔH60+50mmHg	pre	27.59±24.58	18.28±25.52	25.26±24.49
	post	13.44±13.13	13.64±18.20	14.93±17.27
ΔFAO	pre	14.81±28.25	16.85±27.23	20.07±26.05
	post	10.85±11.19	8.99±24.73	15.79±20.39

Mean±standard deviation

Table 4.12: The effect of treatment on DBP by group and arm

DBP (mmHg)		External control (n=14) Non-dominant	Training (n=18) Non-dominant	Training (n=16) Dominant
ΔH60+50mmHg	pre	22.28±21.72	8.86±20.42	20.04±16.21
	post	16.99±11.25	17.38±13.62	15.17±11.52
ΔFAO	pre	12.29±25.01	9.82±22.60	14.43±17.31
	post	12.55±13.02	7.79±17.92	12.13±12.34

Mean±standard deviation

4.3.4 Other Measurements

Participants did improve their training time from the first week (average training time: 17±5minutes) to the last week (20±2minutes, the training time was limited to 20 minutes per session, $p<0.05$). Additionally, participants reported an average RPE of 13±2 the first week and 15±8 the last week of training (not significant).

CHAPTER 5 DISCUSSION

The purpose of this study was twofold: (1) to examine the cardiovascular response to an acute bout of handgrip exercise in as much as it may be reflective of stimulation of the EPR; and (2) to evaluate the influence of non-dominant handgrip exercise training on cardiovascular response to exercise and post-exercise forearm occlusion. The results indicate that we were successful in establishing a model with cardiovascular responses consistent with stimulation of the EPR. With respect to the influence of exercise training, we hypothesized that exercise training would result in attenuated HRV and blood pressure responses to exercise (H60+50mmHg) and recovery (FAO) in the trained arm. Exercise training did not result in an attenuated HRV and blood pressure response to H60+50mmHg and FAO.

5.1 Participant Characteristics

Our participants were well matched for gender, age, height, weight, forearm circumferences, and MVC such that there were no significant differences in these variables between groups for the pre-test. Additionally there were no significant differences between the dominant and non-dominant arms with respect to MVC and forearm circumferences at pre-test. This is in agreement with Mostoufi-Moab et al. (1998), Somers et al., 1992, and Sinoway et al., 1996 that showed no significant difference in MVc or forearm circumference between dominant and non-dominant arms. Our forearm circumference measurements (Non-dominant arm site 1: 26.58±3.31cm; site 2: 23.13±3.32cm; site 3: 18.86±2.17cm) were consistent with forearm circumferences reported by Sinoway et al., 1996 (Non-dominant arm site 1: 27.6±0.50cm; site 2: 24.5±0.5cm; site 3: 19.4±0.4cm).

5.2 EPR Effect

5.2.1 Respiratory Rate, HRV, and Blood Pressure

Respiratory rate did increase significantly during H60+50mmHg (≈+2.82 breaths per minute). This is consistent with results from Fontana et al. (1994) that found an approximate 4 breath per minute increase in respiratory rate with isometric handgrip activity. In this study, respiratory rate during post-exercise forearm occlusion was not significantly different from SB1. To our knowledge there are no previous studies measuring respiratory rate during forearm occlusion. Due to the possibility that HRV measurement may be effected by a change in respiratory rate, it was subsequently used as a covariate for the EPR effect analyses.

We found approximately a 20 beat per minute increase (Mean R-R≈233.11msec) in heart rate during H60+50mmHg. This was consistent with Victor et al. (1987) which found a 13 beat per minute increase in heart rate during dynamic handgrip exericse at 30% MVC (0.7hz) with 50mmHg on the arm and Mostoufi-Moab et al. (1998) that found a 12 beat per minute increase in heart rate with dynamic handgrip exericse at 25%MVC (0.25hz) with 50mmHg of pressure on the arm. During FAO, mean R-R decreased below H60+50mmHg, but remained significantly elevated above SB1 (≈6beats per minute). This is in contrast to findings by Somers et al. (1992), Iellamo et al. (1999), Fisher and White (1999), Victor et al. (1987), Seals (1989), Victor and Seals (1989), and Strange (1999) which found that heart rate during FAO was not significantly different from SB1. One reason for this discrepancy is the method by which heart rate was measured. In the current study we used the mean R-R interval as a measurement of heart period as opposed to mean heart rate used in the studies listed above. From

previous work in this lab we have determined that the standard error of the mean for Mean R-R interval is less than 56 miliseconds, thereby allowing us to detect small changes in heart period (Kluess et al., 2002). Although not reported as significantly different from SB1, the reported mean differences between FAO and SB1 for the above mentioned studies (2 and 5 beat per minute higher heart rates during FAO), were similar to the magnitude of change reported in this study (≈6 beats per minute),

There have been no previous studies reporting SDNN change during H60+50mmHg. However, the SDNN at SB1 was similar to a previous study in this lab using similar participant demographics and tachogram time period for analysis (Kluess et al., 2002). Additionally, the magnitude of change in SDNN with H60+50mmHg in the current study was 1.5-fold greater than in our previous study where there was no forearm occlusion during exercise (Kluess et al., 2002). SDNN returned to SB1 levels during FAO which was an expected change considering that SDNN calculated for less than five minutes is believed to more strongly reflect changes in parasympathetic activity (Task force, 1996, Kluess et al., 2000).

For the variables LFnu and MAP the following discussion will focus on the behavior of these variables in reference to the hypotheses regarding cardiovascular responses consistent with stimulation of the EPR and comparison to other literature.

Post-exercise occlusion is commonly employed to quantify the EPR (McClosky and Mitchell, 1972; Victor et al., 1987; Hayward, Wesselmann, Rymer, 1991; Somers et al., 1992; Seals, 1989; Victor and Seals, 1989; Fisher and White, 1999; Strange, 1999). The theory is that if EPR plays a significant role in the muscle sympathetic and cardiovascular responses to exercise, then occluding the blood flow to the muscle after

cessation of the exercise will result in a persistent elevation of muscle sympathetic activity and blood pressure due to continued stimulation of group IV afferents by the metabolites trapped in the muscle (Victor and Seals, 1989).

This effect has been demonstrated in both isometric and dynamic exercise using afferent, efferent, and end-organ models (Seals, 1989; Somers et al., 1992; Victor and Seals, 1989). Our finding that MAP was elevated above SB1 during FAO is consistent with the idea that our exercise protocol (H60+50mmHg) sufficiently stimulates the EPR. However, we also expected to see an elevation in LFnu, an index of sympathetic activity, during FAO. Despite an elevation of LFnu during H60+50mmHg, LFnu did not remain elevated above SB1 during FAO.

The present findings initially appear to stand in contrast to Iellamo et al. (1999) who found a persistent elevation in low frequency power (ms^2) and low frequency normalized units during post-exercise occlusion. Interestingly, however, the change from spontaneous breathing (LFnu=63.8%) to exercise (LFnu=82.8%) in Iellamo et al. (1999) was similar to that of the present study (pre-train non-dominant arm LFnu SB1=53.43%; H60=73.62%). The statistical significance observed in Iellamo, et al. (1999) might have been aided by their use of isometric single-leg exercise, while the present study employed dynamic handgrip exercise. The use of a larger muscle mass and an isometric challenge may have provided a greater stimulus for an EPR response.

The effect of muscle group size on sympathetic activity has been previously studied by Seals (1993) who showed that the MSNA, heart rate, and blood pressure responses to isometric handgrip exercise were greater than that for isometric finger flexion exercise, despite relative intensity and perceived effort being the same. This

relationship between muscle group size and magnitude of cardiovascular and sympathetic response to exercise also holds for dynamic exercise. Victor et al. (1987) had their participants perform dynamic handgrip exercise at 30%MVC which caused a 5-b/min increase in heart rate and a 7-mmHg increase in MAP, but no change in MSNA (Victor et al., 1987). However, arm cycling exercise at 30% of max resulted in a 32-b/min increase in heart rate, a 12-mmHg increase in MAP, and a 25 burst/minute increase in MSNA (Victor and Seals, 1989).

Further, when these findings are paired with post-exercise occlusion to specifically target the EPR, leg activity (dynamic or isometric) always results in persistent elevation of blood pressure during post-exercise occlusion (Iellamo et al., 1999; Fisher and White, 1999; Strange, 1999). However, dynamic handgrip exercise only induces persistent elevation of blood pressure when the intensity is at least 60%MVC (1hz contraction speed) or the forearm is occluded during the activity (Victor and Seals, 1989; Victor et al., 1987). Therefore, the muscle group exercised appears to play a role the cardiovascular and autonomic response to exercise and the ability of HRV to detect these changes.

The mode by which this change in signals from parasympathetic withdrawal during activity to an increase in parasympathetic activity during recovery, despite continued EPR signaling, is not entirely understood. It is known that dynamic exercise increases the activity of both group III and IV afferents, however, group III afferents are mostly inactive during post-exercise occlusion.

Work by Potts and Mitchell (1998) suggests that the movement detection (group III afferents) aspect of the EPR influences baroreceptor resetting during exercise and

therefore, when that stimulus is removed the barorecepetor reflex acts to slow heart rate. Regardless of the specific mechanism, it is plausible that since the skeletal muscle vasculature does not contain parasympathetic innervation, MAP remains high as a consequence of heightened sympathetic activity. However, in the heart, where vagal activity has a dramatic chronotropic effect, spectral parameters of HRV may not provide a clear measure of sympathetic changes. This is particularly true during periods where both sympathetic and parasympathetic activity is elevated above the baseline (or comparison condition). The reason for this is that LFnu is calculated as LF power in relation to the change in HF power. Therefore a decrease in LFnu during FAO can indicate any one of the following: 1) an increase in HF with no change in LFpower; 2) an increase in HF with a decrease in LF power, or 3) an increase in the power of both HF and LF power such that the magnitude of change in HF power is greater than the change in LF power.

As mentioned, the present investigation involved dynamic exercise, while Iellamo et al. (1999) employed isometric work. It has been well established that isometric exercise induces greater sympathetic, heart rate, and blood pressure response compared to dynamic activity at the same workload (Gonzalez-Camarena et al., 2000). However, there is evidence that the cardiovascular and autonomic response to isometric and dynamic exercise is not the same. Gonzalez-Camarena et al. (2000) compared the HRV response of isometric (30%MVC) and dynamic (30 to 60%VO_2max, 60 rpm, 6 min) quadriceps femoris exercise. They controlled for both exercise intensity (%MVC) and MAP at peak exercise and found that dynamic exercise resulted in an overall reduction in HRV power (ms^2) and a shift toward the LF domain indicating that

the exercise induced parasympathetic withdrawal and an increase in sympathetic modulation of the heart. However, isometric exercise resulted in an overall increase in HRV power (ms^2) with both an increase in LF and HF power, indicating that there was an increase in parasympathetic and sympathetic modulation of the heart. They concluded that the cardiovascular responses to isometric and dynamic exercise are not controlled through the same mechanisms and speculated that the baroreceptor reflex may play a greater role in isometric compared to dynamic exercise. This conclusion is substantiated by Potts and Mitchell (1998) who found that baroreceptor resetting during exercise is influenced by the movement detection aspect of the EPR and therefore, baroreceptor resetting may not occur to the same degree with a single isometric contraction, compared to multiple dynamic contractions.

Differences in the muscle group exercised, exercise intensity, and exercise mode all appear to alter the relative contribution of the different cardiovascular control mechanisms during exercise and forearm occlusion. However, it also may be possible to alter cardiovascular control mechanisms while controlling for the above variables. In a previous study, our participants performed handgrip exercise at 60%MVC without 50mmHg (Kluess et al., 2001). Interestingly, the change in LFnu from rest to H60 with 50mmHg of forearm occlusion (Train non-dominant pre: 19.33%) were approximately the same as our previous study where the participants performed H60 without forearm occlusion (Kluess et al., 2001, control day: 21.20%). In the previous study (Kluess et al., 2002), MAP did not remain elevated above SB1 during FAO, while in the current study, this effect did occur. This finding is important and, according to EPR theory,

suggests that forearm occlusion during exercise resulted in greater EPR activation than the exercise alone.

Adreani and Kaufman (1998) using a decerebrate cat model have previously demonstrated the effect of occlusion during exercise. They found that group III and IV afferents were directly stimulated with simulated walking activity with and without complete arterial occlusion. However, the magnitude of change in group III and IV afferent firing frequency to activity with arterial occlusion was 44% and 47% higher, respectively, than simulated walking without arterial occlusion.

In humans, exercise with reduced metabolite washout has also been used to induce EPR activity. Mostoufi-Moab et al. (1998) used dynamic handgrip exercise at 25%MVC with and without 50 mmHg of pressure on the arm and found no elevation of blood pressure with 25%MVC alone, but found a significant elevation in blood pressure when occlusion was present.

Victor et al. (1987) demonstrated the EPR specificity of this maneuver in humans. They performed dynamic handgrip exercise at 30% MVC with 50mmHg of pressure on the arm and found that it resulted in approximately three-fold greater heart rate and blood pressure response, compared to exercise with no pressure on the arm. Additionally, they found a persistent elevation in blood pressure and MSNA during forearm occlusion. This did not occur when dynamic handgrip exercise at 30%MVC was performed without occlusion (Victor and Seals, 1989). They specifically attributed the cardiovascular and muscle sympathetic nerve activity responses to greater stimulation of the EPR through increased trapping of metabolites during the activity.

Therefore, the contrast between the HRV findings from Illeamo et al. (1999) and the current study are most likely explained by differences in muscle group size where a larger muscle mass induces an EPR response that persists more completely into forearm occlusion. Evidence from previous studies in this lab and others suggest that despite similar increases in sympathetic activity during exercise it is possible to shift the relative contribution of cardiovascular control mechanisms through trapping metabolites during exercise, altering the mode of exercise, and exercising different muscle groups (Iellamo et al., 1999; Gonzalez-Camarena et al, 2000; Kluess et al., 2002).

5.2.2 Associations Among Variables

It has been well established that the magnitude of the change in sympathetic activity and, to some degree, the cardiovascular response to activity is related to the muscle group recruited. This inference has come primarily from studies that have compared different muscle groups (e.g., arms vs. legs). However, there is no information concerning the relationship between muscle mass within the same muscle group and cardiovascular and autonomic response to exercise. Additionally, the relationship between muscle mass and the cardiovascular response, as assessed during post-exercise forearm occlusion has never been formally reported.

The working hypothesis for the relationships stated above is that a larger muscle mass contains more group III and IV afferents and therefore, the afferent signal is larger and signals a greater magnitude cardiovascular response than a smaller muscle mass (Seals, 1993; Victor et al., 1987; Victor and Seals, 1989; Fisher and White, 1999; Iellamo et al. 1999; Gonzalez-Camarena et al. 2000; Somers et al. 1992; Strange, 1999). Therefore we used linear regression to infer if a relationship existed among

circumference site 1 (the site closest to the olecranon process) and the change from SB1 to H60+50mmHg and the change from SB1 to FAO for the variables Mean R-R, SDNN, LFnu, and MAP.

We found a negative relationship between arm circumference and change in Mean R-R from SB1 to H60+50mmHg. This was accompanied by a positive relationship between arm circumference and increase in MAP from rest to H60+50mmHg (dominant arm, pre-test). Both of these relationships are consistent with the above stated hypothesis and suggest that a larger muscle mass (as inferred from arm circumference) induces a greater cardiovascular response to exercise (of a given relative intensity).

However, we found a positive relationship between forearm circumference and the change in Mean R-R from forearm occlusion to SB1 (dominant arm). Additionally, we found a positive relationship between MAP during forearm occlusion and forearm circumference (dominant and non-dominant arm). Therefore, a person with a larger arm circumference recovers to a higher heart period during forearm occlusion than people with a smaller arm circumference. The finding that cardiovascular recovery (mean R-R) is more rapid with a larger arm circumference suggests that central command plays a large role in the cardiovascular response to exercise, compared to a smaller forearm circumference.

Although not previously explored, there is some evidence supporting this supposition. Training studies in the arm have demonstrated that training adaptations are localized to the arm used and attributed to changes in EPR (Somers et al., 1992; Mostoufi-Moab et al., 1998; Sinoway et al., 1996). However, studies using leg training

(single leg and both legs) have found significant changes in central command, but no detectable changes in EPR (Fisher and White, 1999; Seals and Chase, 1989). Although it is difficult to directly relate these chronic studies to the current acute study it does set a precedent for larger muscle masses having a greater influence on central command.

In contrast to Mean R-R, MAP remained higher with a larger muscle mass and is consistent with the hypothesis that larger muscles have greater magnitude change consistent with the EPR effect. The association between forearm circumference and the behavior of Mean R-R and MAP during H60+50mmHg and FAO hypothetically indicate that the EPR and central command are not entirely redundant mechanisms and under certain circumstances can act in opposition to each other.

Uniquely, we found that the cardiovascular response to exercise and forearm occlusion in a single muscle group is consistent with the idea that cardiovascular response is subject to significant variation in the predominant mechanism of cardiovascular control despite maneuvers to emphasize a single mechanism. These findings further underscore the complexity of studying mechanisms of cardiovascular control in humans.

5.3 The Effect of Training

The second part of this study was to evaluate the influence of non-dominant handgrip exercise training on cardiovascular response to exercise and post-exercise forearm occlusion. As expected, we saw no MVC, forearm circumference, blood flow, respiratory rate, HRV, or blood pressure changes in the external control group (non-dominant arm) and the dominant arm of the training group.

However, the data did not support our hypothesis that exercise training would result in attenuated HRV and blood pressure responses to exercise and FAO in the trained arm. While it would be tempting to speculate that perhaps the training was not sufficient to evoke an adaptation *per-se*, it should be noted that a reduction in vascular resistance during RHBF in the trained arm was evident after the training period.

5.3.1 Participant Characteristics

There was no significant effect of training on MVC or forearm circumference at any of the measured sites. This is consistent with results from Alomari et al. (2001), Somers et al. (1992), Mostoufi-Moab et al. (1998), and Sinoway et al. (1996).

5.3.2 Blood Flow and Vascular Resistance

We hypothesized that RHBF would be significantly higher following handgrip exercise training in the non-dominant arm. In contrast to studies by Alomari et al. (2001); Sinoway, Shenberger, Wilson, McLaughlin, Musch, Zelis (1987); and Green, Cable, Fox, Rankin, Taylor (1994) we did not see a significant change in RHBF following exercise training. However, when a paired T-test was run on the trained arm (non-dominant), vascular resistance during RHBF was lower following the training period. While providing only a weak inference, such a training adaptation would be consistent with previous published data from our laboratory (Alomari et al., 2001).

5.3.3 Respiratory Rate, HRV, and Blood Pressure

There was no significant effect of training for respiratory rate and SDNN for ΔH60+50mmHg or ΔFAO. To our knowledge, there are no previous studies concerning the changes in respiratory rate and SDNN under conditions similar to the current study. While we did see a trend towards a training adaptation in SDNN, this parameter is

primarily thought of as a marker of parasympathetic activity, and therefore it is not likely to be addressing EPR *per-se.* Additionally, there was no training effect with Mean R-R during either ΔH60+50mmHg or ΔFAO. This finding is in agreement with studies by Mostoufi-Moab et al. (1998), Sinoway et al. (1996), and Somers et al. (1992) which also found no difference in mean R-R at rest and during exercise.

In contrast to our hypothesis, the response of LFnu was not attenuated during either ΔH60+50mmHg or ΔFAO following training. As discussed previously, our HRV measurements under the conditions used during this study were complicated by two main factors: a) the high vagal activity in the heart dampening the ability to see peripheral sympathetic changes and b) a previously unreported association between arm circumference and predominant cardiovascular mechanism complicated interpretation of the data. As a result of these problems it was highly unlikely that we would see the hypothesized attenuation of HRV response to the conditions.

Additionally, we calculated the type II error rate for LFnu during ΔH60+50mmHg or ΔFAO (see appendix F). This indicated that we had sufficient power to detect changes with ΔH60+50mmHg, but with ΔFAO we had a 52% chance of a type II error. The elevated type II error rate was likely due to nearly significant pre-test differences. Regardless, this finding does not interfere with the inference that exercise training did not attenuate the response in LFnu (See appendix F, tables F.3 and F.4).

However, in agreement with others, we did not see attenuation in HRV response to the conditions in the dominant arm (Somers et al., 1992; Alomari et al., 2001; Sinoway et al., 1996). No change in the dominant arm, despite training in the non-dominant arm, does suggest that central command was not altered by our handgrip

exercise training protocol. This finding is in contrast to Fisher and White (1999) that found a change in central command using single leg training. These disparate findings may be the result of differences in cardiovascular control by muscle group and size.

Despite the problems experienced with HRV variables we observed an EPR effect during FAO in MAP and expected to see an attenuation of MAP response with training. However, we observed no main effect of treatment for MAP. A post-hoc calculation of the type II error rate revealed that we had a 34.7% chance of committing a type II error on the treatment by group interaction (see appendix F). However, this slightly elevated type II error rate does not weaken our inference about a lack of a training effect in that the mean responses in MAP in the trained arm (non-dominant) were very close in magnitude (Pre-test: 18.17mmHg; post-test: 19.21mmHg). No MAP changes with handgrip exercise training is in contrast to studies by Sinoway et al. (1996) and Mostoufi-Moab et al (1998) that report a decreased MAP during handgrip exercise testing. There are several differences between the current study and those by Sinoway et al. (1996) and Mostoufi-Moab et al. (1998) including the intensity of the handgrip exercise, the length of time of the exercise bout, and the contraction speed.

The exercise intensity used during training and during testing in studies by Mostoufi-Moab et al. (1998) and Sinoway et al. (1996) were much lower than that used in the current study. However, Mostoufi-Moab et al. (1998) and Sinoway et al. (1996) did have participants exercise to fatigue during the training sessions, while our participants were limited to 20 minutes per training session. Despite the limitation in time for our subjects, the initial exercise intensity was 2-fold greater than Sinoway et al. (1996) and Mostoufi-Moab et al. (1998) and the first week most participants were

fatigued (drop in force produced of more than 10 units) in under 20 minutes (average training time for the first week was17±5 minutes). Additionally, the reported average rating of perceived exertion for the first week of training was 15 (Hard) and for the last week was 17 (very hard). These data, although weak, suggest that our training protocol was fatiguing and perceived as effortful. Therefore, it is difficult to explain the differences in MAP results among studies exclusively to training intensity and exercise session duration

Another difference between this study and previous studies is the contraction/relaxation speed during the training. Sinoway et al. (1996) and Mostoufi-Moab et al. (1998) used 2-second contraction to 3-second release for exercise training and found a decrease in MAP during exercise testing. However, studies such as Somers et al. (1992) and the current study used 1-second contraction and 1-second release and found no change in MAP during exercise testing.

The mechanism by which this contrast occurs is not readily apparent and given the minimal number of studies it is difficult to attribute attenuation of MAP response with exercise training to contraction/relaxation speed. However, further study regarding the effect of contraction/relaxation speed and cardiovascular response to exercise training is warranted. Therefore, it is difficult to conclusively attribute the differences in the MAP results to either exercise intensity, exercise session time, or contraction/relaxation speed. However, the influence of the factors should be further studied.

5.3.4 Limitations

The findings in this study were limited by a number of factors. One such factor was the difficulty in measuring changes in sympathetic activity in the heart under

conditions where the heart is experiencing a marked increase in vagal activity. This factor limited our ability to measure persistent increase in sympathetic activity during forearm occlusion. Additionally, resolution of sympathetic activity during forearm occlusion may further have been limited by the use of a small muscle group during exercise. It appears that HRV is a suitable measurement of sympathetic activity during forearm occlusion when the leg muscles are used (Iellamo et al., 2000).

Another limitation was the indication that there is an association between forearm circumference and cardiovascular control during exercise and post-exercise forearm occlusion. This finding added significant and previously unappreciated complexity to the model of post-exercise forearm occlusion. This association also potentially limited our ability to see training induced changes since not all participants responded in the same way to exercise.

In addition, it is not clear as to the extent to which environmental factors such as temperature, relative humidity, and sound contribute to the variability of the parameters of interest. Moreover seasonal changes in these parameters have not been fully investigated. As to the former, greater environmental control may eliminate some day to day variation in parameters. As to the latter, however, it would appear outside of a yearlong training program, the influence of seasonal variations would be difficult to exclude.

CHAPTER 6 SUMMARY AND CONCLUSIONS

The purpose of this study was twofold: (1) to examine the cardiovascular response to an acute bout of handgrip exercise in as much as it may be reflective of stimulation of the EPR; and (2) to evaluate the influence of non-dominant handgrip exercise training on cardiovascular response to exercise and post-exercise forearm occlusion. The cardiovascular responses observed in this study were consistent with the hypothesis that the EPR was stimulated during exercise. However, the resolution of HRV measurements during post-exercise forearm occlusion was complicated by the large chronotropic influence of vagal activity on the heart. Uniquely, we found that the cardiovascular response to exercise and forearm occlusion in a single muscle group is subject to significant variation in the predominant mechanism of cardiovascular control despite maneuvers to emphasize a single mechanism. These findings further underscore the complexity of studying mechanisms of cardiovascular control in humans.

With respect to the influence of exercise training, we hypothesized that exercise training would result in attenuated HRV and blood pressure responses to exercise (H60 +50mmHg) and post-exercise forearm occlusion (FAO) in the trained arm. However, the data do not support this hypothesis. Additionally, we did not see the hypothesized increase in reactive hyperemic blood flow following exercise training. However, using a less rigorous statistical test we did see a decrease in forearm vascular resistance during the reactive hyperemia condition. This finding is in agreement with other studies using single arm handgrip exercise training and suggests that the training protocol did induce a physiological adaptation.

In conclusion, we were successful in using HRV to measure autonomic changes during exercise. The failure of HRV to measure persistent sympathetic activity expected during post-exercise forearm occlusion suggest that HRV may not be a good tool to use with small muscle exercise in subjects with apparently healthy cardiovascular control mechanisms. However, it is possible that this model will provide good resolution during post-exercise forearm occlusion in populations, such as heart failure patients or the elderly, where autonomic function is compromised.

One very important finding of this study was the association between forearm circumference and cardiovascular control. This association, particularly during post-exercise forearm occlusion, requires further study and possibly requires a re-evaluation of the cardiovascular control mechanisms involved in post-exercise forearm occlusion.

Although limited, we did see a decrease in vascular resistance in the trained arm and an increase in endurance time of the training sessions suggesting that a training effect did occur in our participants. However, the training effect observed could not be linked to a change in EPR.

Further work needs to be done to more completely characterize EPR under conditions of exercise and post-exercise forearm occlusion before these conditions can be used conclusively to indicate changes in EPR both acutely and chronically. Additionally, the adaptability of the EPR has not been conclusively shown in either handgrip or leg exercise training and requires further study.

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APPENDIX A IRB FOR STUDY

LSU INSTITUTIONAL REVIEW BOARD (IRB)

IRB APPLICATION: APPROVAL OF PROJECTS WHICH USE HUMAN SUBJECTS

The IRB uses this form to obtain succinct answers to questions it must consider. If incomplete, your application will be returned! You can download this form and all other IRB documents from http://www.osr.lsu.edu/osr/irb) & complete it with your wordprocessor (use Courier 12 and make all margins 0.6" to fit). Call Dr.Robert C. Mathews for assistance, 578-8692)

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(IRB Use: IRB# _____ Review Type: Expedited__ Full ___)

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1. Principal Investigator: Robert H. Wood Rank: Ph.D. Dept.: Kinesiology Ph: (225) 578-9142 E-mail: rwood@lsu.edu SSN (confidential**)121-60-1457

Co-investigators*: Heidi A. Kluess, M.S.

*Student? Y/N __ Thesis/dissertation/class project? Y/N ___

Dept.: Kinesiology Ph:(225) 578-4983

E-mail:hklues1@lsu.edu

SSN 595-62-7782 (Confidential - For database indexing)**

2. Project Title: The Effect of a Single Dose of Aspirin on Cardiovascular Responses During Handgrip Exercise and Forearm Occlusion following 6 weeks of exercise training

3. Proposed duration (months): 12 Start date: September 1, 2001

4. Funding sought from: no funding sought

5. LSU Proposal #: _____ 6. Number of subjects requested: 50

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A. ASSURANCE: PRINCIPAL INVESTIGATOR (named above)

I accept personal responsibility for the conduct of this study (including ensuring compliance of co-investigators/co-workers in accordance with the documents submitted herewith and the following guidelines for human subject protection: The Belmont Report, LSU's Assurance with OPRR, and 45 CFR 46 (Available from OSR or at http://www.osr.lsu.edu/irb/)

Signature of PI _____ Date _____

B. ASSURANCE OF STUDENT/PROJECT COORDINATOR named above

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature _____ Date _____

Protocol Abstract

The role of Aspirin in the secondary prevention of myocardial infarction is well-established (Elwood et al., 1998). The steering committee of the Physician's Health Study Research Group (1989) reported a 44% reduction in the risk for myocardial infarct in individuals randomized to Aspirin. Although the mechanism for the reduction in risk of myocardial infarct is not entirely understood, Aspirin

has a powerful antithrombic effect. Aspirin rapidly inhibits prostaglandin formation in blood platelets by inactivating fatty acid cyclooxygenase. As a result of this inhibition, Aspirin prevents the synthesis of thromboxane A_2, a vasoconstrictor and platelet activator. Thus, Aspirin therapy appears to prevent platelet aggregation and subsequent thrombosis.

However, along with the blockade of platelet cyclooxygenase, Aspirin also blocks the synthesis of prostaglandins in vascular tissue. Prostacyclin, a prostaglandin, is a potent vasodilator and platelet inhibitor. Aspirin therapy inactivates platelet cyclooxygenase for the life of the platelets (approximately 10-12 days) (Ali, 1995). In contrast, vascular tissues can resynthesize cyclooxygenase after exposure to Aspirin, and thus regain the capacity to form prostaglandins. The ability of vascular tissue to resynthesize cyclooxygenase is, in part, dependent on the dose of Aspirin administered. Evidence suggests that Aspirin given in doses less than 325 mg/day is insufficient to block prostaglandin synthesis (Gajados et al., 1998, Physician's Health Study Research Group 1989, Aspirin Myocardial Infarction Study Research Group, 1980). Therefore, vascular reactivity should be preserved, in contrast to doses exceeding 325 mg/day. Interestingly, there is some evidence that Aspirin and related non-steriodal anti-inflammatory drugs (NSAIDs) in high doses (50 mg per kg of body weight) attenuates HR and blood pressure responses to exercise by blocking chemically sensitive receptors in the interstitium of the skeletal muscle (Fontana et al., 1994; Rotto et al., 1990). Combined, the reduced vascular reactivity and blunted

hemodynamic response to exercise may result in reduced blood flow to tissue and subsequently contribute to symptoms of exercise intolerance and fatigue.

In light of these studies, attempts have been made to maximize the beneficial effects in antithrombic therapy using alternative regimes like low dose Aspirin, alternate day Aspirin, and control release Aspirin (Physician's Health Study Research Group, 1989; Elwood et al., 1998). Interestingly, data is lacking on the effect of Aspirin therapy on cardiovascular function, such as heart rate, blood pressure, and blood flow. Knowledge regarding the effects of Aspirin on cardiovascular function may be clinically useful in further determining the efficacy in patients with or at risk for occlusive vascular disease. This lab has previously established a dynamic handgrip exercise protocol through which heart rate and blood pressure responses are predictable (Kluess et al., 2000) and reliable (Wood et al., 2000). In Spring 2001 we conducted a study concerning the effect of a single dose of Aspirin on acute cardiovascular and blood flow response to exercise. We found that 650 mg of Aspirin decreased heart rate response to both rest and forearm occlusion, but did not influence blood pressure response or blood flow.

It is well established that exercise training results in a decrease in both heart rate and blood pressure response to exercise (Sinoway et al, 1996; Seals and Chase, 1989; Levy et al., 1998; Fisher and White, 1999; Somers et al, 1992). However, the mechanisms controlling decreased heart rate and blood pressure response to exercise are not well understood. To date only one longitudinal study has been performed to evaluate the role of skeletal muscle afferents in

cardiovascular control following exercise training (Somers et al., 1992). They found a significant decrease in sympathetic activity during exercise and forearm occlusion, but no difference in heart rate and blood pressure response following low intensity forearm training. Since their exercise training stimulus was below that which is understood to induce skeletal muscle afferent activity it is possible their mixed results were due to inadequate training stimulus (Victor and Seals, 1989). Forearm occlusion both during and following exercise is a potent and well established method to measure the effect of skeletal muscle afferents on cardiovascular response (Victor, et al., 1987, Somers, 1992, Iellamo, 1999, Fisher and White, 1999, Victor and Seals, 1989).

Therefore, the purpose of this study is to compare the effect 650-mg of Aspirin on heart rate, blood pressure, and blood flow responses to rest, handgrip exercise and recovery during forearm occlusion prior to and following 6 weeks of exercise training with forearm occlusion. Such data may provide further information regarding hear rate and blood pressure control during exercise.

The specific aims of the study are:

1) to determine the effect of 650-mg of Aspirin on cardiovascular response(HR, BP, and blood flow) to rest, exercise, and recovery during forearm occlusion prior to exercise training;

2) to determine the effect of 650-mg of Aspirin on cardiovascular response(HR, BP, and blood flow) to rest, exercise, and recovery during forearm occlusion following exercise training;

Participation in this study will not be of any particular benefit to the participant. Rather, it is the purpose of this investigation to further our knowledge of how the human body adapts to changes in the environment.

a) A maximum of 50 volunteers over the age of 18 will be asked to participate in this study. All volunteers will be healthy adults without any over signs such as diabetes, heart disease, hypertension, emphysema, gastrointestinal disturbances or bleeding or gynecological problems. Individuals with any current medical problems (including those listed above), current infection, or on medication known to affect cardiovascular function will be excluded from the study. Additionally, anyone that has taken aspirin within two weeks prior to testing will be excluded.

b) All participants will receive a comprehensive explanation of the proposed study, its benefits, inherent risks, and expected commitments with regard to time. Following explanation of the proposed study, all participants will be required to read and sign the informed consent document during the first visit and prior to any experimentation.

Session 1: Informed consent will be obtained from each participant. The participant will undergo an initial risk screening using the Health Status Questionnaire that requires the participant to answer questions regarding his health history and history of physical activity. The participant will be measured for height and weight. Each participant will be placed in a supine position for 20 minutes prior to evaluation of forearm vascular blood flow.

Forearm blood flow measurements will be obtained from the dominant arm using an EC-5R plethysmography system (Hokanson, 1995). Prior to the experiment blood pressure cuffs will be positioned around the participant's upper arm and wrist, and a mercury-in-silastic strain gauge placed around the forearm approximately 10 cm distal to the olecranon process. The forearm will be extended and slightly supinated and supported by a Styrofoam block. Immediately before the blood flow measurements, hand circulation will be occluded for 1 minute by inflating the cuff at the wrist to 240 mmHg. All blood flow measures will be made with an upper arm venous collecting pressure of 50 mmHg and reported in milliliters per minute per 1000 milliliters of tissue.

Following the resting blood flow measure, the participant will be asked to perform a handgrip exercise task preceded and followed by quiet rest. The handgrip exercise tasks consist of squeezing a handgrip measuring device once every two seconds at less than maximal effort while the cuff on the upper arm is inflated at 50 mmHg for 5 minutes. Lastly, reactive hyperemia following forearm occlusion will be achieved by inflating the cuff on the upper arm to 200 mmHg for 3 minutes. Blood flow measures will then be determined as described above. Throughout the testing procedures, heart rate and blood pressure will be recorded. The participant will then be allowed to sit up and asked to ingest 650-mg of aspirin. One hour after oral administration of Aspirin, the participant will be asked to repeat the protocol described above using the opposite arm. The testing procedures for both arms will last approximately 150 minutes.

Session 2: The participant will arrive at the lab at least 48 hours following session

1. The protocol for session 1 will be followed except the order of the arms tested will be

opposite to session 1.

Exercise Training: Participants will report to the laboratory 4 days per week for 6 weeks. The participant will be asked to perform a handgrip exercise to volitional fatigue. The handgrip exercise task consists of squeezing a handgrip measuring device once every two seconds at less than maximal effort while the cuff on the upper arm is inflated at 50 mmHg for 5 minutes. Each training session will last approximately 30 minutes. Session 3: Following 6 weeks of exercise training the participants will perform the protocol outlined in session 1.

Session 4: At least 48 hours after session 3, the participants will be asked to perform the protocol outlined in session 2.

d) There is no risk associated with either the blood flow or exercise testing involved in this study. However, some participants may experience temporary discomfort when the blood pressure cuffs are inflated and exercise can result in muscle soreness, stiffness, or tightness or in some rare cases muscle injury. There is some risk of gastrointestinal disturbances and bleeding with administration of Aspirin. However, in studies that have used 650 mg of Aspirin/day or less report a very low incidence of adverse events. (CARS investigators, 1997).

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GENERAL QUESTIONS:

PROVIDE ANSWERS TO FOLLOWING QUESTIONS. EITHER LOAD INTO WORDPROCESSOR AND INSERT RESPONSES OR ATTACH SHEETS AND REFERENCE ALL ITEMS; ANY INAPPLICABLE ITEMS MAY BE SKIPPED ONLY BY LISTING THE QUESTION LETTER WITH N/A. (BY SIGNATURE, PAGE 1, YOU ARE ATTESTING TO THE ACCURACY OF THE RESPONSES TO THESE QUESTIONS.)

A. Why is the use of human subjects necessary (v.s. animals/in vitro)

The question addressed is specific to the value of cardiovascular response in human participants.

B. Specify sites of data collection.

Data will be collected in room 20 of the Gym Armory and/or room 54 of the Long Fieldhouse under the direction of Dr. Robert Wood, Assistant Professor, LSU, Department of Kinesiology.

C. If surgical or invasive procedures are used, give name, address, and telephone number of supervising physician and the qualifications of the person(s) performing the procedures. Comparable information when qualified participation or supervision is required or appropriate.

N/A

D. Provide the names, dosage, and actions of any drugs or other materials administered to the subjects and the qualifications of the person(s) administering the drugs.

Aspirin 650 mg

E. Detail all the physical, psychological, and social risks to which the subjects may be exposed.

There are no risks associated with electrocardiography, and non-invasive arterial tonometry. There are no health risks associated with the evaluation of forearm blood flow and handgrip exercise, although participants may experience mild discomfort during either. Lastly, there are minimal risks associated with aspirin administration.

1) Plethysmography/non-invasive blood flow assessment

There is no risk associated with venous occlusion plethysmography. However, participants may experience some temporary discomfort during the five minutes of forearm occlusion as a result of the 240 mmHg applied to the upper arm. Should the participant ask to discontinue this procedure then the upper-arm cuff will be deflated immediately.

2) Handgrip exercise

Because of the small amount of muscle mass involved in this type of exercise, the typical cardiovascular responses involve a modest increase in HR, suggesting that this activity is of no greater than moderate in intensity, and therefore it is appropriate for all individuals, and poses extremely low health risk for the study sample. Handgrip exercise applied according to the methods described may result in mild muscle soreness, muscle stiffness, or tightness, or in some rare cases muscle injury.

3) Aspirin Administration

Although rare, allergic reaction may occur with a single dose of aspirin. While, chronic use of Aspirin at 650 mg or less, can result in gastrointestinal disturbances and bleeding (0.74 events per 100 people studied). As is the case in this study, the incidence of such adverse reactions is even less in studies with short-term exposure (CARS investigators, 1997).

F. What steps will be taken to minimize risks to subjects?

The risk associated with handgrip is extremely low, nonetheless proper patient screening careful exercise prescription, and appropriate assessment of exercise response will ensure that the risks are kept minimal. In addition, the investigator will have graduate level training in clinical exercise physiology and will possess current CPR certification. The risk associated with aspirin administration will be minimized by careful evaluation of Aspirin sensitivity while recruiting subjects. Additionally, anyone that has taken aspirin within two weeks prior to testing will be excluded.

G. Describe the recruitment pool (community, institution, group) and the criteria used to select and exclude subjects.

Participants for this study will be recruited from undergraduate and graduate courses in the LSU department of Kinesiology. A total of 50 volunteers over the age of 18 will be asked to participate in this study. All volunteers will be healthy adults without any overt signs such as asthma, diabetes, heart disease, hypertension, emphysema, gastrointestinal disturbances or bleeding, known allergy to Aspirin, or gynecological problems. Individuals with any current medical problems (including those listed above), current infection, or on

medication known to affect cardiovascular function will be excluded from the study.

H.List any vulnerable population whose members are included in this

project (e.g., children under the age of 18; mentally impairedpersons; pregnant women; prisoners; the aged.)

High risk individuals (as defined above) will be excluded from participation in this study.

I. Describe the process through which informed consent will be obtained.(Informed consent usually requires an oral explanation, discussion, and opportunity for questions before seeking consent form signature.)

All participants will receive a comprehensive explanation of the proposed study, its benefits, inherent risks, and expected time commitments. Following the explanation of the proposed protocol, all participants will be allowed a period of questioning. Individuals who are willing to participate in the study will be required to read and sign the informed consent document during the first visit. Each participant will receive a copy of the informed consent form.

J. (1) Is this study anonymous or confidential? (Anonymous means that the identity of the subjects is never linked to the data, directly, or indirectly through a code system.) (2) If a confidential study, detail how will the privacy of the subjects and security of their data will be protected.

There is no conflict of interest for the investigators in this protocol. The only potential gain to the investigators is the professional benefit from academic publication or presentation of the results. The names and personal information of

each participant will not appear in print or will be presented in a manner that would not identify the individual. All documents containing confidential information will be kept in a locked cabinet in a locked laboratory. Only members of the research team whose names are indicated herein will be granted access to these records.

CONSENT FORM

1. Study Title: The Effect of a Single Dose of Aspirin on Cardiovascular Responses During Handgrip Exercise and Forearm Occlusion Following 6 Weeks of Exercise Training

2. Performance Site: Department of Kinesiology, Louisiana State University

3. Investigators: The following investigators are available for questions at the telephone numbers listed below:

Principal Investigator:

Robert H. Wood, Ph.D.

Department of Kinesiology (225) 578-9142

Co-Investigators:

Heidi A. Kluess, M.S.

Department of Kinesiology (225) 578-2036

4. Purpose of the Study: The purpose of this study is to compare the effect one dose of Aspirin on heart rate, blood pressure, and blood flow during: 1) rest, 2) less than maximal muscular effort, and 3) during inflation of a blood pressure cuff on the participant's upper arm to 240 mmHg for 2 minutes. These measures will be made before and after 6-weeks of arm exercise.

5. Participant Inclusions: A total of 50 volunteers over the age of 18 will be asked to participate in this study. All volunteers will be healthy adults without any over signs such as diabetes, heart disease, high blood pressure, lung disease, digestive disturbances or bleeding, or female reproductive problems.

6. Participant exclusions: Individuals with any current medical problems (including those listed above), current infection, or on medication known to affect heart or blood vessels will be excluded from the study. Additionally, anyone that has taken aspirin within two weeks prior to testing will be excluded.

7. Description of the Study: The proposed study consists of 4 separate visits to the laboratory over a 4-month period and 24 sessions of exercise training. Each visit is expected to last approximately 2.5 hours. After informed consent is obtained, the participant will be asked to answer questions regarding his health history, history of physical activity, and will be measured for height and weight.

Pre-test Session 1: Each participant will be placed in a supine position and asked to squeeze a handgrip-measuring device as tightly as possible. Following 20 minutes of quiet rest, forearm blood flow measurements will be obtained. Prior to the experiment blood pressure cuffs will be positioned around the participant's upper arm and wrist, and a band placed around the forearm to measure blood flow. Before blood flow measurements are taken, the blood pressure cuff on the wrist will be inflated to 240 mmHg for 1 minute. Following this, the upper arm cuff will be inflated to 50 mmHg for approximately 10 seconds and a blood flow measurement taken.

Following 5 minutes of quiet rest, the cuff on the participants upper arm will be inflated to 50 mmHg and they will be asked to perform a handgrip exercise task consisting of squeezing the hand grip measuring device once every two seconds at less than maximal effort to fatigue. Immediately following the handgrip exercise bout, the cuff on the upper arm will be inflated to 200 mmHg for 2 minutes. Blood flow measures will then be determined as described above. During rest, the performance of the handgrip exercise task, and forearm occlusion the measurements of heart rate, heart rhythm, and blood pressure will be obtained using a blood pressure measuring device on the wrist. The participant will then be asked to ingest 650 mg of Aspirin. Following one hour of quiet rest, the opposite arm of the participant will be testing using the protocol described above. The testing procedures will last approximately 150 minutes.

Pre-test Session 2: The heart rate, blood pressure, and blood flow responses to handgrip exercise and cuff inflation will be tested using the protocol described in session 1, but the order of the arms tested will be opposite to session 1.

Exercise training: Participants will be asked to perform 16 sessions of handgrip exercise training over a 6-week period. The participant will be asked to perform a handgrip exercise task consisting of squeezing the hand grip measuring device once every two seconds at less than maximal effort while the cuff on the upper arm is inflated to 50 mmHg. Each session will last approximately 30 minutes.

Post test Session 3 and 4: Following 6 weeks of exercise training, participants will be tested using the protocol outlined in sessions 1 and 2 above.

8. Benefits: Participation in this study will not be of any particular benefit to the

participant. Rather, it is the purpose of this investigation to further our knowledge of how the human body adapts to changes in the environment.

9. Risks: There are no known risks associated with obtaining heart rate, heart rhythm, and blood pressure. The risks associated with this protocol can be broken down into risk associated with the evaluation of forearm blood flow, the handgrip exercise testing component, and Aspirin administration.

a) Plethysmography/Non-invasive blood flow assessment

There is no health risk associated with the procedures involved in this study. However, participants may experience some temporary discomfort when the blood pressure cuff is inflated prior to the measurement of forearm blood flow.

b) Handgrip exercise testing and training

The risk of handgrip exercise testing using the methods described are minimal in this population, but include muscle soreness, muscle stiffness or tightness, or in some rare cases muscle injury. The risk of this study will be minimized as a result of proper evaluation, education, and treatment, careful handgrip exercise prescription, and appropriate assessment of handgrip exercise responses, the presence of well trained personnel capable of monitoring handgrip exercise equipment, and a person trained in CPR available while subjects are being tested.

c) Aspirin administration

Although rare, allergic reaction may occur with a single dose of Aspirin. Regular use of Aspirin can result in digestive disturbances and bleeding. However, studies that have used daily doses of 650 mg of Aspirin or less report a very low

chance of allergic reaction and digestive disturbances (0.74 events per 100 people studied). The incidence of reactions to Aspirin is even less in studies with short-term exposure.

10. Right to refuse: Participation in this study is completely voluntary and participants may withdraw at any time without penalty.

11. Privacy: Though the results of this study may be published, the privacy of the participants will be protected and their identities will remain confidential.

12. Release of Information: The records for each participant in this study may be reviewed by investigators, but participant identity will be kept confidential. The data collected will only be used for purposes stated in this consent form.

13. Financial Information: There is no monetary cost to participate in this study nor will any payment be rendered to participants. The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Chairman, LSU Institutional Review Board, (225)388-8692. I agree to participate in the study described above and acknowledge the researchers obligation to provide me with a copy of this consent form if signed by me.

_____ _____

Participant's signature **date**

_____ _____

Witness signature **date**

_____ _____

Investigator's signature **date**

APPENDIX B MEDICAL HISTORY QUESTIONNAIRE

Fall 2001

Participant Medical History

The effect of a single dose of aspirin on cardiovascular responses during handgrip exercise and forearm occlusion on 4 weeks of exercise training

Name : _____ Date: _____ Phone number: _____ Age: _____ Gender: Male Female Race:

Cauc. Afr.Am other _____

Height: _____ Weight: _____

Has the participant ever been diagnosed with:

	Yes	No
CVD		
Hypertension	____	____
Diabetes	____	____
Emphysema	____	____
Asthma	____	____
Hyperlipidemia	____	____
Epilepsy	____	____
Heart Failure	____	____
Osteoarthritis	____	____

Rheumatoid Arthritis ____

____

Peripheral Vascular Disease ____

____

Anemia ____ ____

Signs or symptoms of hypoglycemia ____

____

Surgery on arms or hands ____ ____

24 Hour History:

How much sleep did you get last night? (Please circle one)

1 2 3 4 5 6 7 8 9 10

(Hours)

How much sleep do you normally get? (Please circle one)

1 2 3 4 5 6 7 8 9 10

(Hours)

When did you last have:

A cup of coffee or tea _____

Eat food _____

Smoke a cigarette, cigar, or pipe _____

Aspirin, ibuprofen, or other drugs _____

Drink alcohol _____

Any recent illness _____

Exercise _____

Describe your general feelings today. (Circle one) Excellent Very Good Good

Neither bad nor good Bad Very bad Very, very bad Terrible

APPENDIX C SUPPLEMENTAL DIAGRAMS FOR METHODS

Diagram C.1: Set up for pre- and post- testing

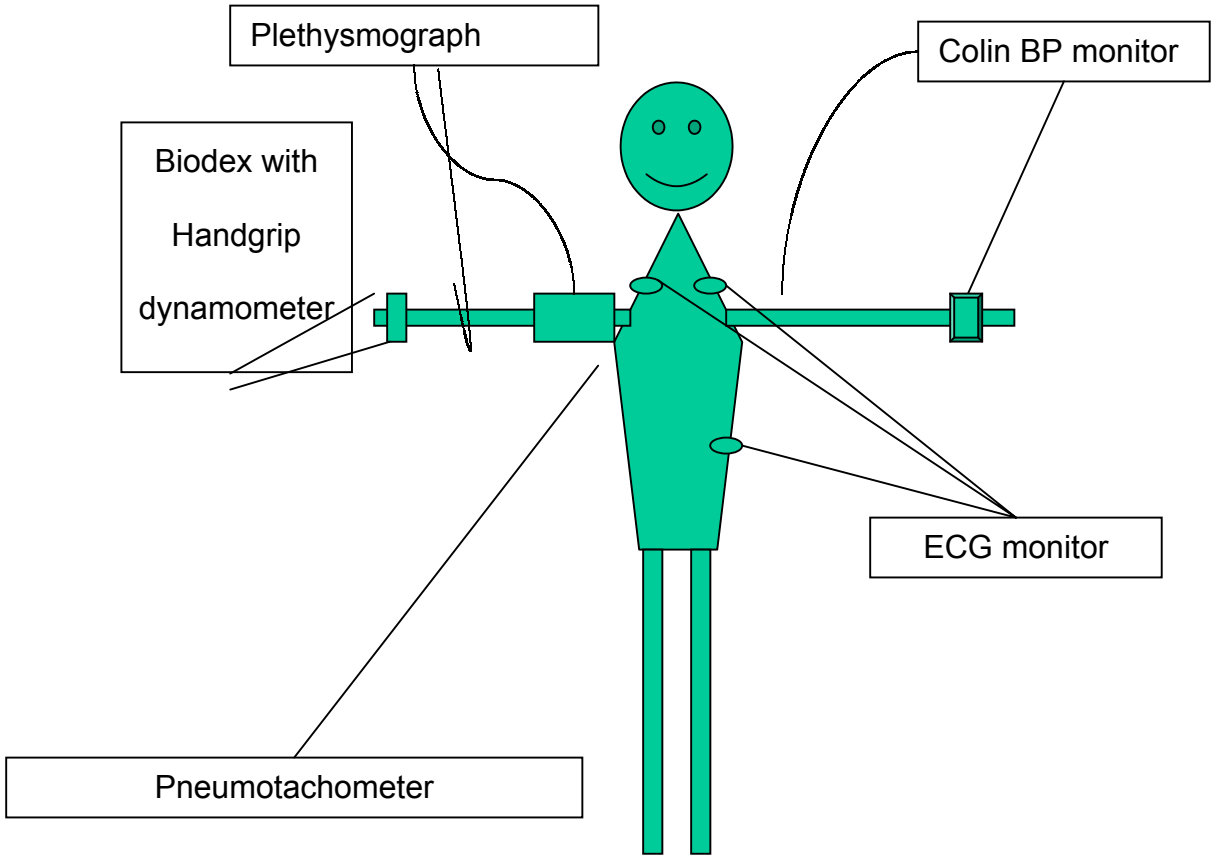
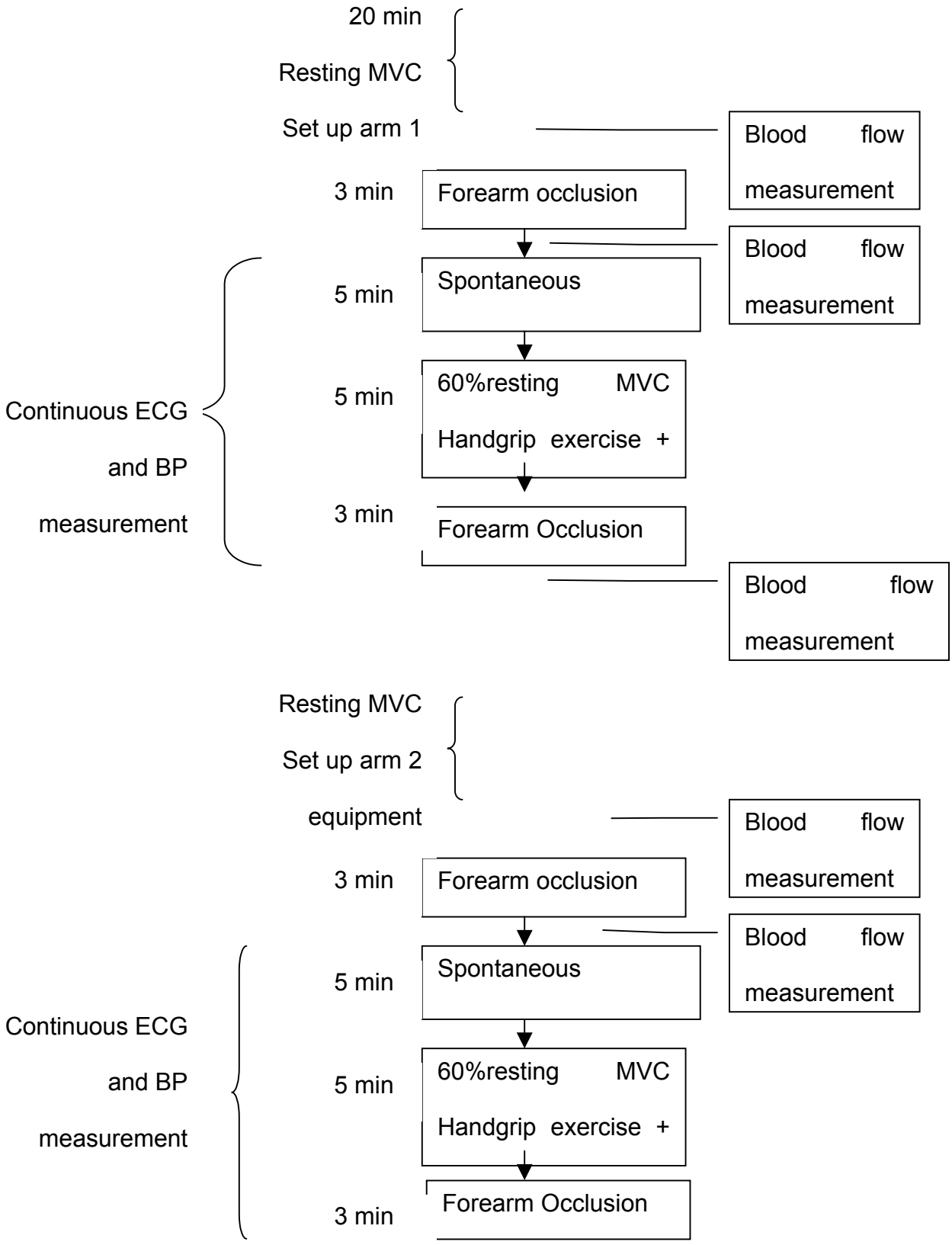


Diagram C.2: Data collection time line



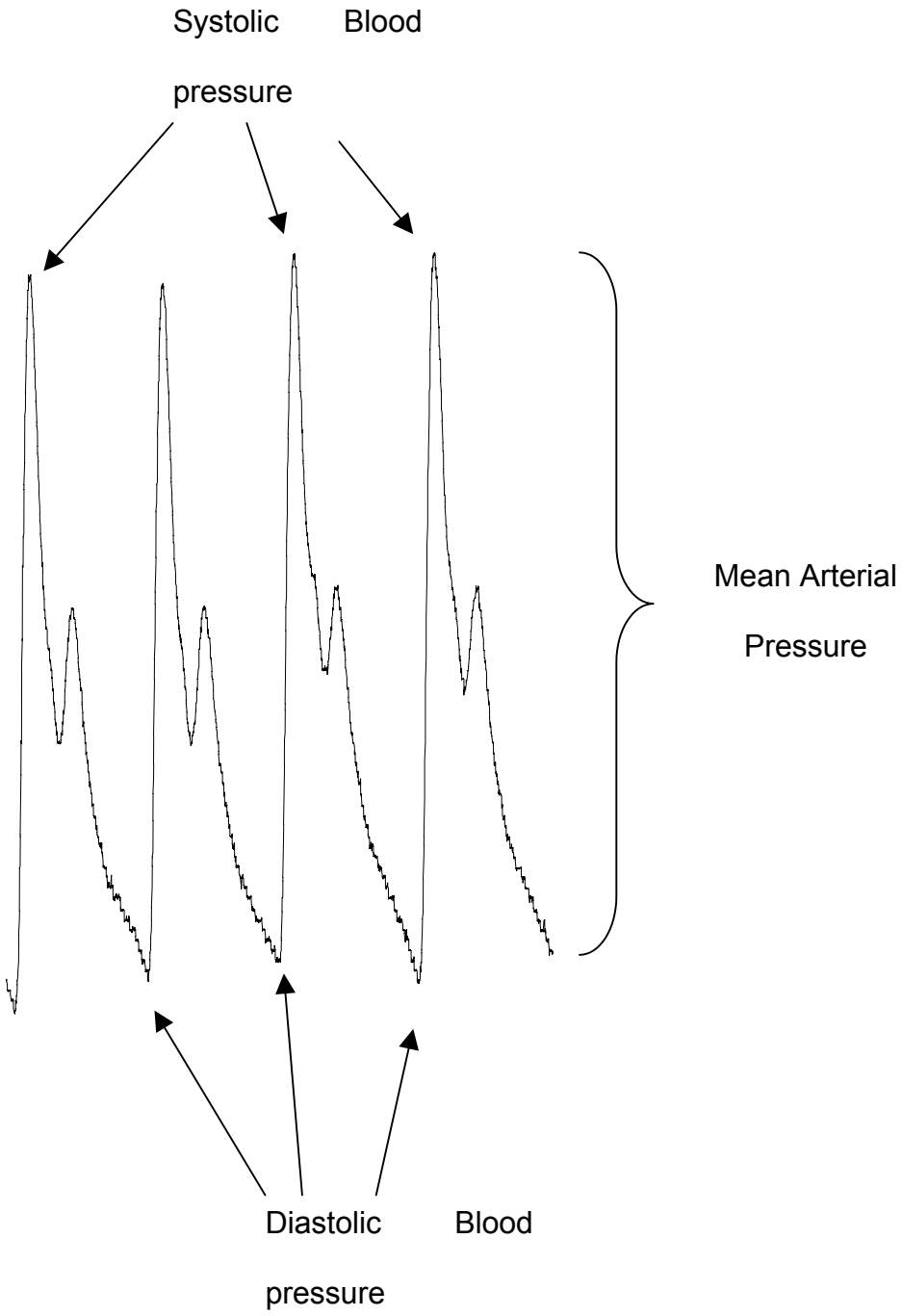


Diagram C.3: Example of blood pressure wave with labels for systolic and diastolic blood pressure

APPENDIX D FIGURES FOR THE INDIVIDUAL RESPONSES IN THE TRAINING GROUP IN THE NON-DOMINANT ARM DURING H60+50MMHG AND FAO PRE-TRAINING AND POST TRAINING FOR MEAN R-R, LFNU, AND MAP

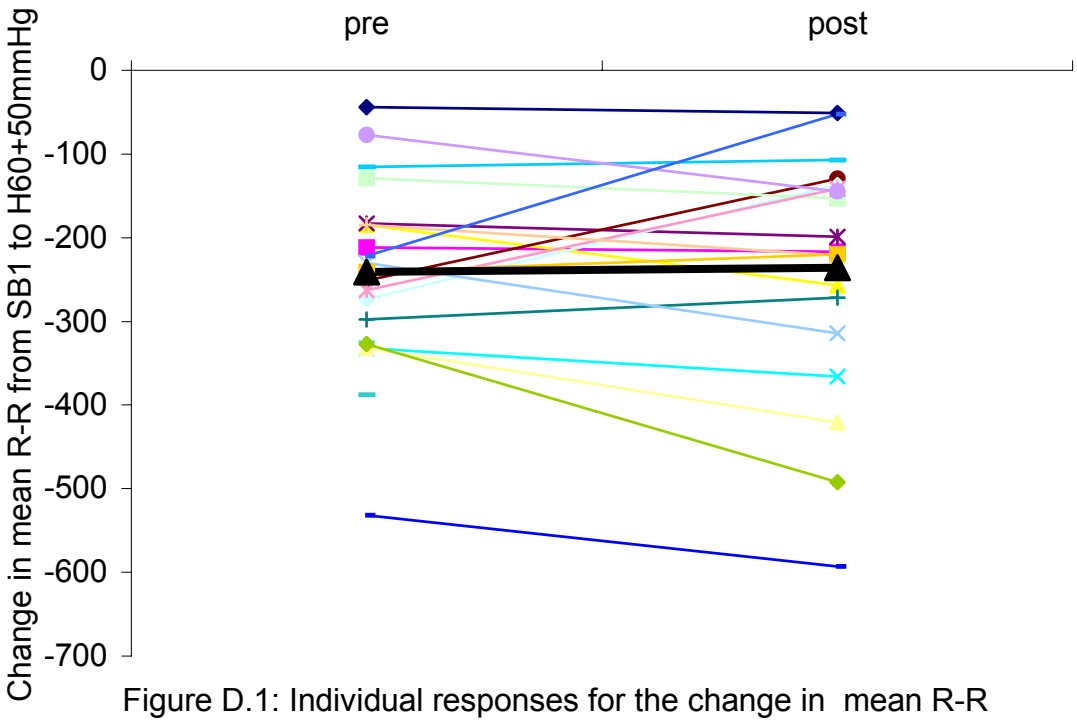


Figure D.1: Individual responses for the change in mean R-R (msec) from SB1 to H60+50mmHg before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

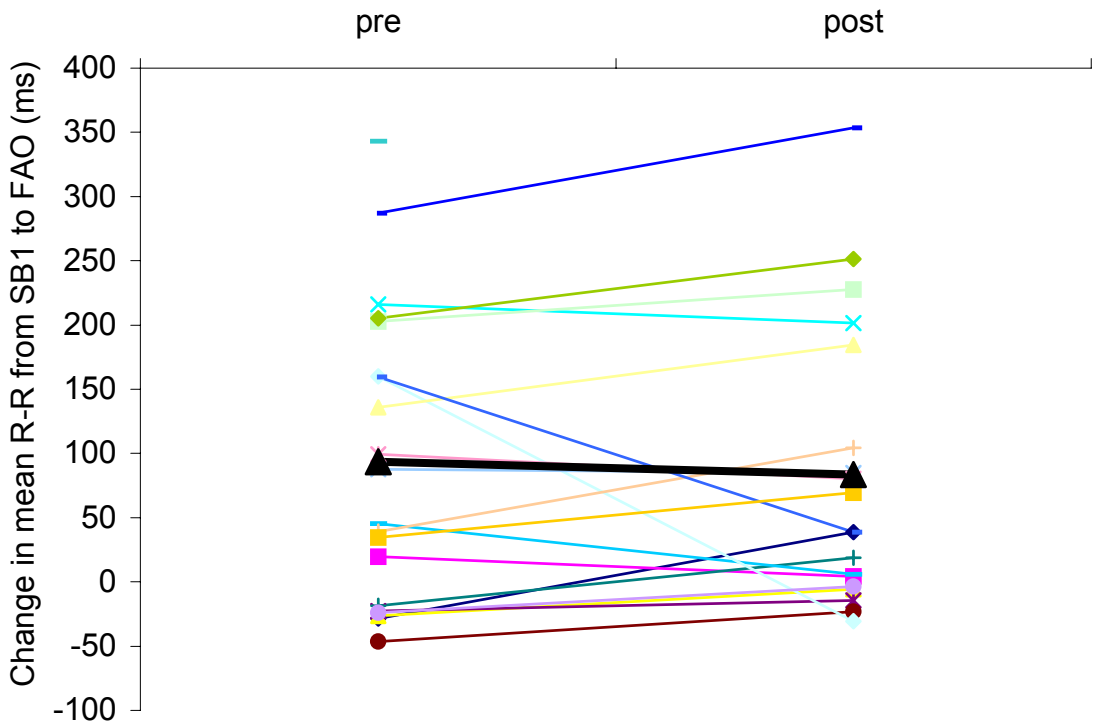


Figure D.2: Individual responses for the change in mean R-R from SB1 to FAO before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

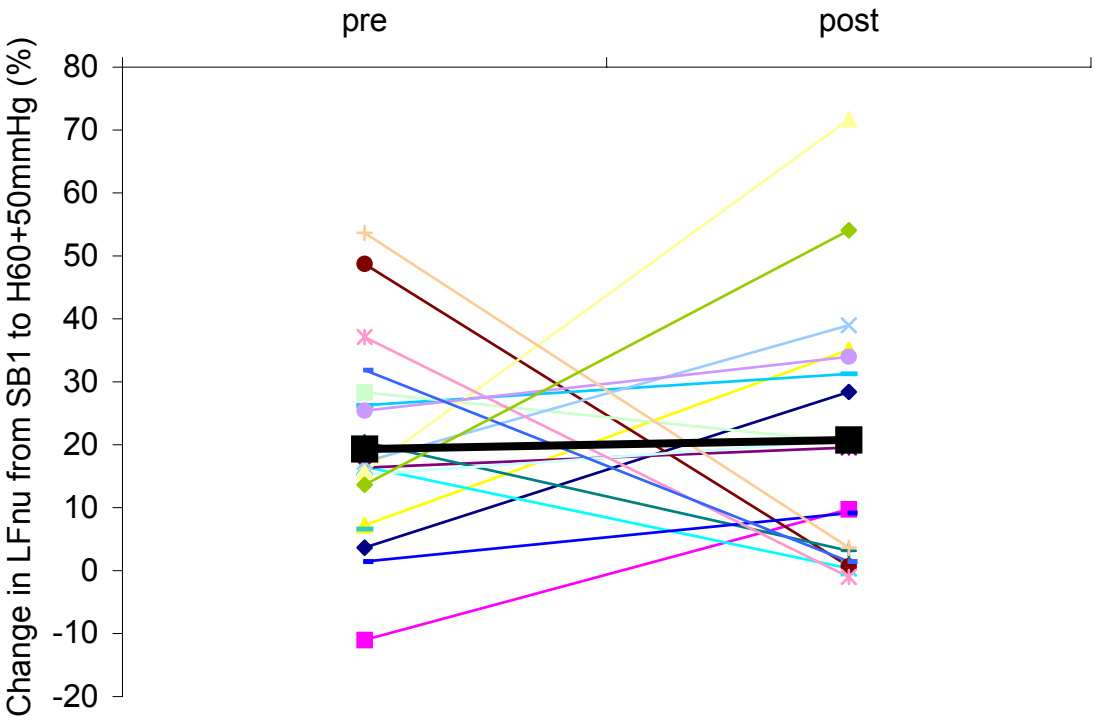


Figure D.3: Individual responses for the change in LFnu from SB1 to H60+50mmHg before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

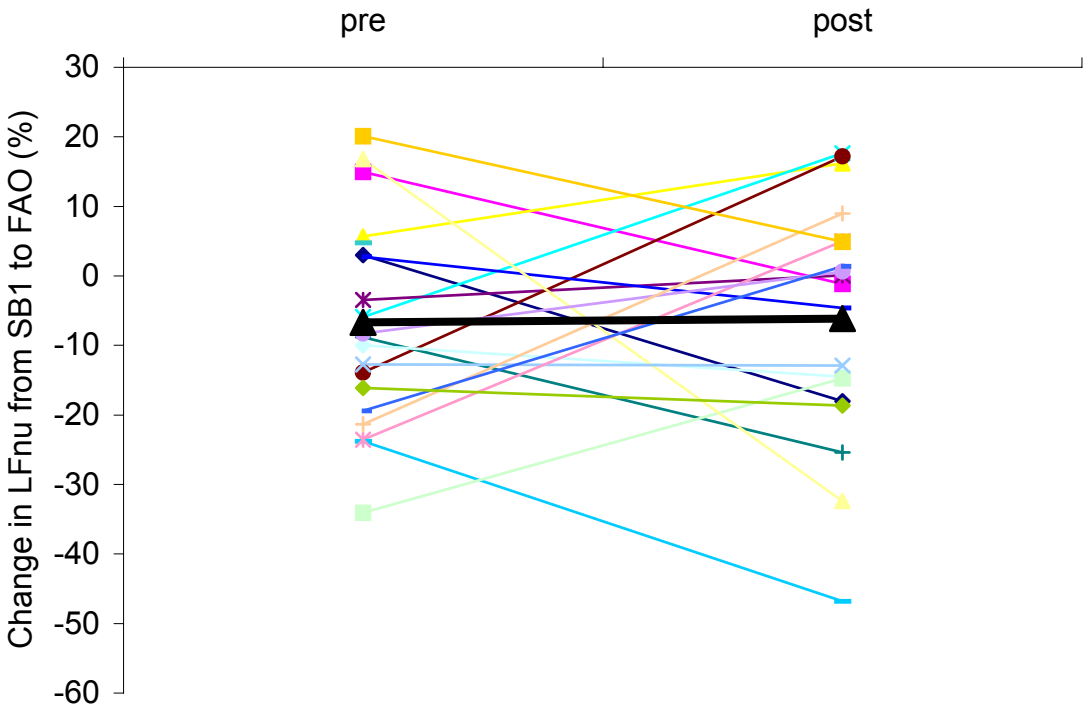


Figure D.4: Individual responses for the change in LFnu from SB1 to FAO before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

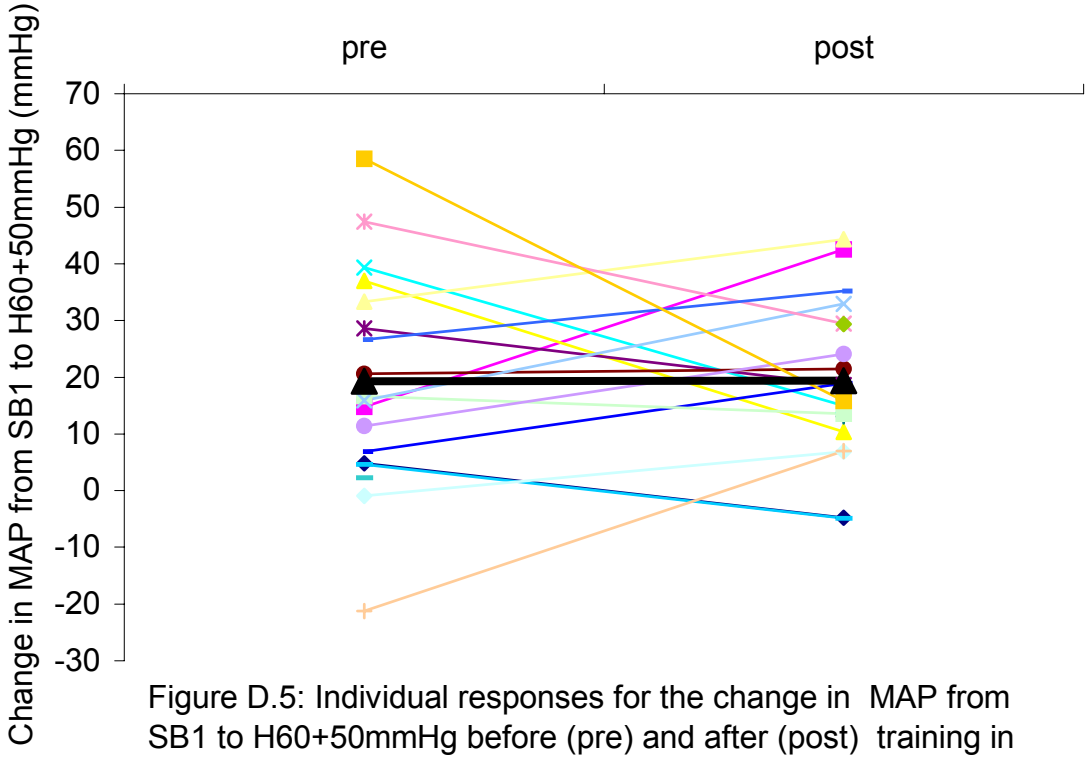


Figure D.5: Individual responses for the change in MAP from SB1 to H60+50mmHg before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

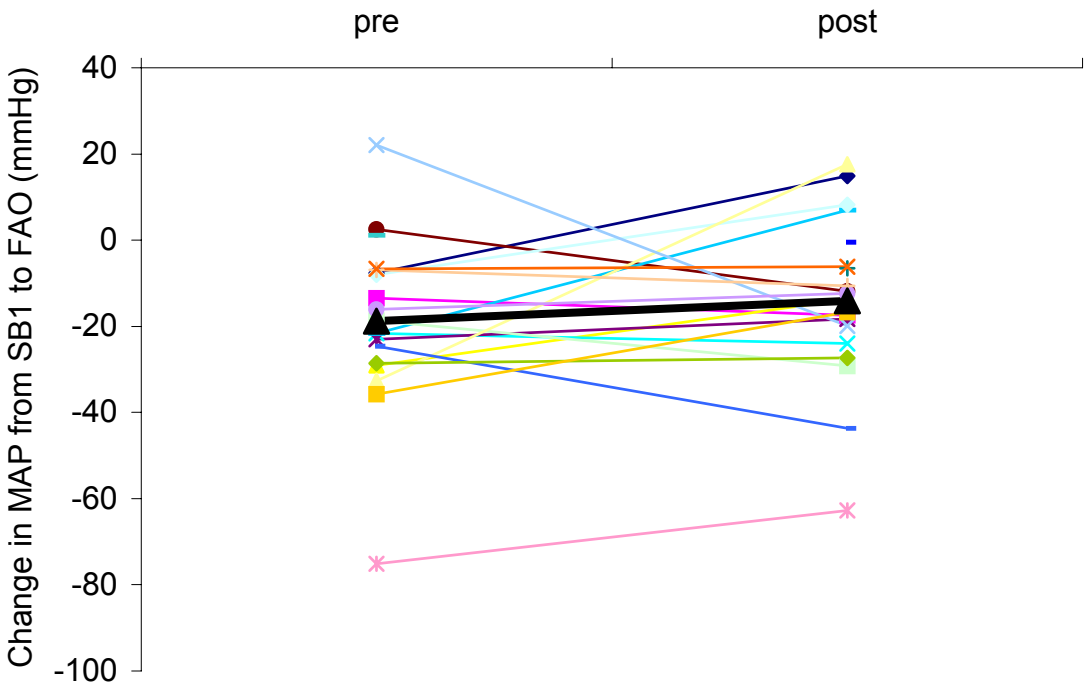


Figure D.6: Individual responses for the change in MAP from SB1 to FAO before (pre) and after (post) training in the non-dominant arm of the training group . The bold line indicates the mean of the group.

APPENDIX E ABSOLUTE VALUES FOR POST-TEST

Table E.1: The effect of condition on respiratory rate by group and arm (post-test only).

Respiratory rate (breaths/min)	External control (n=14) Non-dominant	Trained (n=17) Non-dominant	Internal control (n=17) Dominant
SB1	16.79±2.83	16.59±3.59	16.11±3.97
H60+50mmHg	20.29±5.59	20.59±5.95*	19.88±5.36*
FAO	14.71±5.36H	15.00±4.35H	15.33±3.50H

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

Table E.2: The effect of condition on mean R-R by group and arm (post-test only).

Mean R-R (miliseconds)	External control (n=15) Non-dominant	Trained (n=19) Non-dominant	Internal control (n=19) Dominant
SB1	972.62±122.56	980.95±190.20	998.76±178.86
H60+50mmHg	751.29±85.10*	744.77±114.95*	775.23±95.36*
FAO	912.88±149.68H	897.51±144.80H	948.34±150.79H

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

Table E.3: The effect of condition on SDNN by group and arm (post-test only).

SDNN (miliseconds)	External control (n=15) Non-dominant	Trained (n=19) Non-dominant	Internal control (n=19) Dominant
SB1	101.44±46.76	87.76±36.77	103.21±47.28
H60+50mmHg	49.01±25.83	46.76±40.14*	53.61±35.36*
FAO	103.83±71.10	96.99±47.85H	102.84±52.98H

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

Table E.4: The effect of condition on LFnu by group and arm (post-test only).

LFnu (%)	External control (n=15) Non-dominant	Trained (n=19) Non-dominant	Internal control (n=19) Dominant
SB1	56.34±16.57	55.44±22.46	61.89±19.49
H60+50mmHg	69.91±20.61	76.18±13.33*	70.55±16.18*
FAO	57.95±20.62	61.60±17.50H	51.55±17.10H

Mean±standard deviation
H different from H60
* different from SB1

Table E.5: The effect of condition on MAP by group and arm (post-test only).

MAP (mmHg)	External control (n=14) Non-dominant	Trained (n=19) Non-dominant	Internal control (n=16) Dominant
SB1	80.08±8.72	78.27±20.95	78.41±12.26
H60+50mmHg	97.55±14.40*	97.69±23.63*	92.85±13.67*
FAO	95.17±14.27*	92.33±29.14*	91.44±17.28*

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

Table E.6: The effect of condition on SBP by group and arm (post-test only).

SBP (mmHg)	External control (n=14) Non-dominant	Trained (n=16) Non-dominant	Internal control (n=16) Dominant
SB1	121.67±19.69	123.11±19.27	113.48±16.07
H60+50mmHg	135.11±22.07	136.75±23.06*	129.58±19.59*
FAO	132.52±23.06	132.10±31.21	126.65±26.47

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

Table E.7: The effect of condition on DBP by group and arm (post-test only).

DBP (mmHg)	External control (n=14) Non-dominant	Trained (n=18) Non-dominant	Internal control (n=16) Dominant
SB1	69.44±20.33	62.86±19.77	62.56±16.41
H60+50mmHg	80.73±13.55	80.24±22.95*	77.00±13.20*
FAO	78.52±15.47	70.65±28.17	72.23±16.39

Mean±standard deviation
H different from H60+50mmHg
* different from SB1

APPENDIX F POST HOC POWER ANALYSIS

Table F.1: Mean , standard deviation, confidence interval, and type II error rate for MAP during)H60+50mmHg

Group	Treatment	Mean	Standard error	95% confidence interval	95% confidence interval
				Low	High
External Control	pre	28.85	5.63	17.34	40.35
External Control	post	17.47	3.61	10.09	24.84
Training	pre	18.17	5.01	7.72	28.61
Training	post	19.21	3.27	12.51	25.90

Type II error rate for the interaction of Treatment by group = 0.347

Type II error rate for the main effect of Treatment = 0.256

Type II error rate for the main effect of Group = 0.136

Table F.2: Mean , standard deviation, confidence interval, and type II error rate for MAP during)FAO

Group	Treatment	Mean	Standard error	95% confidence interval	95% confidence interval
				Low	High
External Control	pre	16.48	5.76	4.70	28.27
External Control	post	15.09	4.67	5.55	24.64
Training	pre	17.82	5.23	7.12	28.51
Training	post	15.30	4.24	6.64	23.96

Type II error rate for the interaction of Treatment by group = 0.052

Type II error rate for the main effect of Treatment = 0.077

Type II error rate for the main effect of Group = 0.052

Table F.3: Mean , standard deviation, confidence interval, and type II error rate for LFnu during)H60+50mmHg

Group	Treatment	Mean	Standard error	95% confidence interval	95% confidence interval
				Low	High
External Control	pre	16.83	4.42	7.80	25.86
External Control	post	13.32	5.72	1.63	25.01
Training	pre	20.00	3.51	12.82	27.17
Training	post	20.74	4.54	11.45	30.03

Type II error rate for the interaction of Treatment by group = 0.072

Type II error rate for the main effect of Treatment = 0.059

Type II error rate for the main effect of Group = 0.204

Table F.4: Mean, standard deviation, confidence interval, and type II error rate for LFnu during ∆FAO

Group	Treatment	Mean	Standard error	95% confidence interval	95% confidence interval
				Low	High
External Control	pre	8.43	4.86	-1.50	18.36
External Control	post	-0.01	4.97	-10.25	10.06
Training	pre	-7.26	4.02	-15.47	0.96
Training	post	-6.16	4.11	-14.56	2.24

Type II error rate for the interaction of Treatment by group = 0.251

Type II error rate for the main effect of Treatment = 0.168

Type II error rate for the main effect of Group = 0.517

VITA

Heidi A. Kluess received her Bachelor of Science from the Department of Exercise and Sport Sciences from the University of Florida in May, 1995. She continued at the University of Florida to earn a Master of Science in Exercise and Sport Sciences. She then pursued her Doctor of Philosophy degree from the Department of Kinesiology at Louisiana State University and will graduate May 2002. Heidi is involved in several professional organizations including the Southeastern regional chapter of the American College of Sports Medicine and the American College of Sports Medicine. She has published in the American Journal of Physiology, the Journal of the American College of Cardiology, Medicina Sportiva, and Mollecular and Cellular Biochemistry. She has also presented abstracts at the annual conferences for the American College of Sports Medicine and the American Heart Association. Heidi's future plans are to participate in a post-doctoral fellowship at the University of Illinois at Chicago in the Department of Physiology and Biophysics.